# PHYTOSOMAL FORMULATION OF *THUJA OCCIDENTALIS* EXTRACT FOR THE TREATMENT OF WART

*Dissertation submitted to*

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*In partial fulfillment for the degree of*

**MASTER OF PHARMACY**

**IN**

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*by*

**Reg. No: 26102203**

**DEPARTMENT OF PHARMACEUTICS**

**PERIYAR COLLEGE OF PHARMACEUTICAL SCIENCES FOR GIRLS**

**TIRUCHIRAPPALLI - 620 021**

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## CERTIFICATE

This is to Certify that the dissertation entitled **"PHYTOSOMAL FORMULATION OF *THUJA OCCIDENTALIS* EXTRACT FOR THE TREATMENT OF WART** submitted by **Mr.KARTHIK.R [Reg. No :26102203**] for the award of the degree of **"MASTER OF PHARMACY"** is a bonafide research work done by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli under my guidance and direct supervision.

Place : Tiruchirappalli

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# CERTIFICATE

This is to Certify that the dissertation entitled **"PHYTOSOMAL FORMULATION OF *THUJA OCCIDENTALIS* EXTRACT FOR THE TREATMENT OF WART"** done by **Mr.KARTHIK.R [Reg.No: 26102203]** for the award of the degree of **"MASTER OF PHARMACY"** under The Tamilnadu Dr. M.G.R Medical University, Chennai is a bonafide research work performed by him in the Department of Pharmaceutics, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli. The work was performed under the guidance and supervision of **Dr**.**K.Reeta Vijaya Rani**, **M.Pharm., Ph.D.,** Assistant Professor, Periyar College of Pharmaceutical Sciences for Girls, Tiruchirappalli.

This dissertation is submitted for acceptance as project for partial fulfillment of the degree of **"MASTER OF PHARMACY"** in **Pharmaceutics**, of The Tamilnadu Dr. M.G.R Medical University, during May 2012.

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# ABBREVIATIONS

| Abbreviation | Explanation |
|---|---|
| TGA | Thermal Gravimetric Analysis |
| TCM | Traditional Chinese Medicine |
| CCRAS | Central Council for Research in Ayurvedic Science |
| CCIM | Central Council for Indian Medicine |
| CIR | Cosmetic Ingredients Review |
| DSHEA | Dietary Supplement Health Education Act |
| NCCAM | National Centre for Complementary Alternative system of Medicine |
| CAM | Complementary Alternative system of Medicine |
| CCRAS | Central Council for Research in Ayurvedic Science |
| CCIM | Central Council for Indian Medicine |
| FDA | Food and Drug Administration |
| BPH | Benign Prostatic Hyperplasia |
| HPV | *Human Papilloma Virus* |
| DNCB | Dinitrochlorobenzene |
| DEE | Drug Entrapment Efficiency |
| SEM | Scanning Electron Microscopy |
| LPC | Laser Particle Counter |
| USFDA | United States Food and Drug Administration |

# CHAPTER 1

# 1.INTRODUCTION

### Complementary Alternative System of Medicine

There is new life in the soil for every man. There is healing in the trees for tired minds and for our overburdened spirits, there is strength in the leafs, if only we will lift up our eyes. Remember that nature is your great restorer.

**Herbal medicine** -- also called botanical medicine or phytomedicine , refers to using plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine[26]. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in treating and preventing disease.

Plants had been used for medicinal purposes long before. Ancient Chinese and Egyptian papyrus writings describe medicinal uses for plants. Indigenous cultures (such as African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (such as Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used. Researchers found that people in different parts of the world tend to use the same or similar plants for the same purposes.

In the early 19th century, when chemical analysis first became available, scientists began to extract and modify the active ingredients from plants. Later, chemists began making their own version of plant compounds and over time, the use of herbal medicines declined in favor of drugs.

Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care. In Germany, about 600 - 700 plant-based medicines are available and are prescribed by some 70% of German physicians[24]. In the last 20 years in the United States, public dissatisfaction with the cost of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in herbal medicine use.

The use of herbal supplements has increased dramatically over the past 30 years. Herbal supplements are classified as dietary supplements by the U.S. Dietary Supplement Health and Education Act (DSHEA) of 1994[25]. That means herbal supplements -- unlike prescription drugs -- can be sold without being tested to prove that they are safe and effective. However, herbal supplements must be made according to Good Manufacturing Practices.

Herbs may be used together because the combination is more effective and may have fewer side effects. Health care providers must take many factors into account when recommending herbs, including the species and variety of the plant, the plant's habitat, how it was stored and processed and whether or not there are contaminants (including heavy metals and pesticides).

Nearly one-third of Americans use herbs. Unfortunately, a study in the New England Journal of Medicine found that nearly 70% of people taking herbal medicines (most of whom were well educated and had a higher-than-average income) were reluctant tell their doctors that they used complementary and alternative medicine[27].

**Complementary Alternative System of Medicine**

Alternative medicine is a set of diverse traditional medicinal health care systems and practices that are not generally considered as part of conventional or modern medicine.

Alternative medicine is a health care approach that is applied instead of Conventional or Western medicines. Alternative medicinal treatment may include ingesting or applying organic substances (mostly herbs and some minerals) with a special diet plan (mostly vegetables and fruits), that are not normally advised by a physician who practices conventional or modern medicines[33]. The alternative medicines are not only providing treatment for any health condition but also guide us for how to live a healthier life.

**Alternative medicine user's statistics**

Survey results released in May 2004 by the National Center for Complementary and Alternative Medicine (NCCAM) and the National Center for Health Statistics (part of the Centers for Disease Control and Prevention) shows in the United States alone, approximately 36% of adults used some form of CAM therapy during the past 12 months.

British telephone survey by the BBC in 1998 shows about 20% of adults had used alternative medicine in the past 12 months.

Use of alternative medicine among people in developed countries appears to be increasing. A study in 1998 showed that the use of alternative medicine had risen from 33.8% in 1990 to 42.1% in 1997.

Most commonly practiced complementary treatments include: herbalism, yoga, homeopathy, acupuncture/acupressure, meditation, massage, therapeutic touch, aroma therapy, Chinese medicine, dance therapy, music therapy, osteopathy, chiropractic, and naturopathy.

A 2002 survey of US adults 18 years and older conducted by the National Center for Health Statistics (CDC) and the National Center for Complementary and Alternative Medicine indicated that 49.8% had used some form of CAM[35].

A survey in 2005 among Denmark population aged 16 and above shows 45.2 % of them used alternative medicine of form in their life. 22.5 % had used alternative medicine within lost 12 months.

Patients are averse to dangerous side effects of conventional medicines, even antibiotic medications can have potential to cause life-threatening severe allergic reaction in a very few individuals. Also, many medications may cause minor but troublesome symptoms such as cough or stomach upset. In all of these cases, patients are seeking out alternative medicine treatments to avoid these adverse side effects of modern treatments.

NCCAM has developed one of the most widely used classification systems for the branches of complementary and alternative medicine. It classifies complementary and alternative therapies into five major groups, which have some overlap[34].

1. Whole medical systems: cut across more than one of the other groups; examples include Traditional Chinese medicine Naturopathy, Homeopathy, and Ayurveda
2. Mind-body medicine: takes a holistic approach to health that explores the interconnection between the mind, body, and spirit. It works under the premise that the mind can affect "bodily functions and symptoms"
3. Biology-based practices: use substances found in nature such as herbs, foods, vitamins, and other natural substances
4. Manipulative and body-based practices: feature manipulation or movement of body parts, such as is done in chiropractic and osteopathic manipulation
5. Energy medicine: is a domain that deals with putative and verifiable energy fields:

- Biofield therapies are intended to influence energy fields that, it is purported, surround and penetrate the body. No empirical evidence has been found to support the existence of the putative energy fields on which these therapies are predicated.
- Bioelectromagnetic-based therapies use verifiable electromagnetic fields, such as pulsed fields, alternating-current, or direct-current fields in an unconventional manner.

**Complementary Alternative System of medicine users**

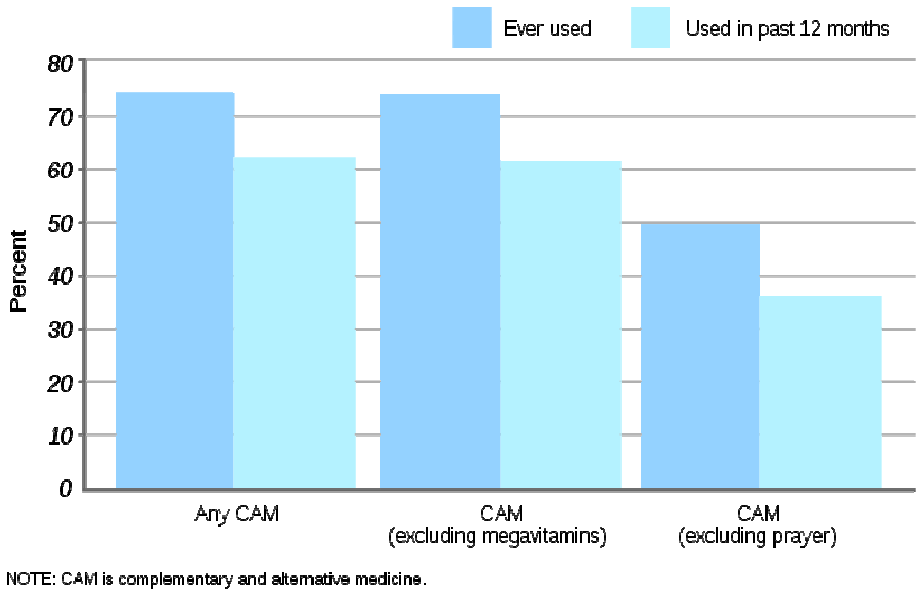


**Figure-1**

## Ancient System of Medicine

All human societies have medical beliefs that provide explanations for birth, death, and disease. Throughout history, illness has been attributed to witchcraft, demons, adverse astral influence or the will of the god.

## 1. Traditional Indian Medicine

The word Ayurveda is derived from 'Ayur', meaning life, and 'veda', meaning knowledge. Ayurveda means the science of life[28]. It is an ancient system of health care and longevity. Ayurveda takes a holistic view of human beings, their health and illness. It aims at positive health, which has been defined as a well-balanced metabolism coupled with a healthy state of being. Disease, according to Ayurveda, can arise from the body and/or mind due to external factors or intrinsic causes.

Ayurvedic treatment is aimed at the patient as an organic whole and treatment consists of the salubrious use of drugs, diet and certain practices. This doctrine was conceived when science was not developed enough to understand even the human body, let alone drug molecules.

Ayurveda, perhaps the most ancient of all medicine traditions, is probably older than traditional Chinese medicine. The origin of Ayurveda is lost in prehistoric antiquity, but its characteristic concepts appear to have matured between 2500 and 500 BCE in ancient India. The earliest references to drugs and diseases can be found in the Rigveda and Atharvaveda, dating back to 2000 BC[31]. Atharvaveda, comprised of 6599 hymns and 700 prose lines, is considered as the forerunner of Ayurveda.

The 'Samhitas,' or encyclopedia of medicine, were written during the postvedic era, and include 'Charak Samhita' (900 BCE), 'Sushruta Samhita' (600 BC) and 'Ashtang Hridaya'(1000 BC). Later on, many more treatises were prepared and the use of medicinal plants is described in 'Nighantu Granthas' between the $7^{th}$ and $16^{th}$ centuries. The most basic concept of Ayurveda is that all living beings derive their subsistence from three essential factors (three doshas), namely vaata, pitta and kapha, which operate in unison. It believes that the human body is composed of living and non-living environments including earth, water, fire, air and space. Illness is the consequence of imbalance between the various elements, and it is the goal of treatment to restore this balance.

Ayurvedic drugs are also attracting much attention for diseases for which there are no or inadequate drugs for treatment in modern medicine, such as metabolic and degenerative disorders[29]. Most of these diseases have multifactorial causation, and there is a growing realization that in such conditions, a combination of drugs, acting at a number of targets simultaneously, is likely to be more effective than drugs acting at one target.

Ayurvedic drugs, which are often multicomponent, have a special relevance for such conditions. For various reasons, Ayurveda has not incorporated much of modern science / scientific tools. Investigation of the biological activity of multicomponent. Ayurvedic drugs will bring Ayurveda into the mainstream of scientific investigations.

**2. Traditional Chinese Medicine**

Traditional Chinese medicine (TCM) has been in practice for more than 200 years and includes acupuncture, massage (tuina), breathing exercise (qi gong) and dietary therapy. TCM has been an integral part of China's healthcare system along with conventional Western medicine. TCM products were safe and effective for the treatment of many human diseases before Western medicine was introduced in China. Famous texts in TCM include the Yellow Emperor's Inner Classic (Hung Di Nei Jing; ~200 BCE to 100 BCE), Divine Husband-man's Classic of Materia Medica (Shen Nong Ben Cao Jing; 25-220 AD) and cold-induced disorders (Shang han Lun; 220 AD).

The most complete reference to Chinese herbal prescriptions is Chinese Materia Medica, published in 1977. It lists nearly 6000 drugs, of which 480 are of plant origin. This ancient system of medicine, believed to be more than 5000 years old, is based on two separate theories about the natural laws that govern good health and longevity, namely 'Yin and Yang', which are in opposition to each other, and the five elements (wu xing).

The five-element theory is similar to the four humours and elements of the Greeks or the three humours of Ayurveda. The five elements are earth, metal, water, wood and fire, each of which is linked to the main organ systems of the body—spleen, lungs, kidney, liver and heart, respectively. It considers that an unbalanced diet, lifestyle or environment will disrupt the body balance, which in turn manifests as symptoms of diseases[29]. The aim of the practitioner of TCM is to restore health by removing the cause, correcting abnormal functioning, opposing the imbalance and normalizing the flow of energy. *Angelica polymorpha* var. sinensis, *Artemesia annua*, *Ephedra sinica*, *Paeonia lactiflora*, *Panax ginseng*, *Rheum palmatum* and *Peuraria lobata* constitute the important medicinal plants of TCM.

**3. Traditional Egyptian Medicine**

Although Egyptian medicine dates from at least 3000 BCE, the last known and most important pharmaceutical record is the Papyrus Ebers (1500 BCE). Use of *Ricinus communis* seeds, *Citrilus colocynthes*, *Senna alexandrina* and *Prunica granatum* roots in large quantities is mentioned in the ancient Egyptian literature. These uses were later documented by the Greek physician Dioscorides (100 CE). Writings of the Greeks, such as Hippocrates (460–377 BCE) and Galen (130–200 CE), also used parts of the Papyrus Ebers. Therefore Greek and ultimately modern, medicine has its origin in Egyptian or Nile Valley civilization.

**4. Traditional Arabic Medicine**

The Babylonians, Assyrians and Sumerians comprise one of the oldest civilizations and several plants were domesticated during this early period. Several medicinal plants are mentioned in civil laws carved on stone and commissioned by the King of Babylon (1700 BCE). The Arabs established drugstores in the eighth century, and the Persian pharmacist Avicenna described all Greco-Roman medicine in his book Canon of Medicine. This text forms the

basis of distinct Islamic healing system known today as Unani-Tibb. *Papaver somniferum* was known to the Sumerians in 4000 BCE as hul gil (joy plant).

The most frequently used medicinal plants in the Middle East are: *Allium cepa, Astracantha gummifera, Carthamus tinctorius, Carum carvi, Ferula asafoetida, Lawsonia inermis, Papaver somniferum, Peganum harmala, Prunus dulcis, Prunica granatum, Salvadora persica, Senna alexandrina, Sesamum indicum, Trachyspermum ammi, Trigonella foenum-graecum* and *Vitis vinifera.*

**5. African, European and Other Traditional Systems of Medicine**

Africa is considered the cradle of Homo sapiens' emergence. Though traditional African medicine is the oldest and perhaps the most diverse of all healthcare systems, detailed documentation on the use of medicinal plants in Africa is lacking. With rapid urbanization, traditional oral knowledge is dwindling fast, e.g. knowledge of traditional oral knowledge of the Khoisan, the Nguni and the Sotho-speaking peoples. Traditional African medicine is holistic, involving both body and mind. Famous African medicinal plants include *Acaccia senegal* (source of gum Arabic), *Aloe ferox, Aloe vera, Artemisia afra, Asplanthus linearis, Boswellia sacra, Catha edulis, Commiphora myrrha, Harpagophytum procumbens,* and *Catharanthus roseus*, etc., Like Africa, South American countries are also rich in biodiversity and diverse healing cultures, but information on the use of medicinal plants is sparse. The famous medicinal plants from this region are *Cinchona pubescens*, *Erythroxylum coca, Ilex paraguariensis, Paullinia cupana, Spilanthes acmella Uncarina tomentosa*. The European healing system is believed to have originated with Hippocrates (460–377 BCE) and Aristotle (384–322 BCE). Subsequent naturalists like Theophrastus (~300 BCE), Dioscorides (100 CE) and the pharmacist Galen (130–200 CE) recorded the use of medicinal plants.

The philosophy was based on the belief that the body is composed of earth, wind, fire and water, similar to the Indian system. The famous book De Materia

Medica by the Greek physician Dioscorides was the standard reference in Europe for more than 1000 years. The use of herbal teas and decoctions is still very popular in Europe, e.g. tea prepared from *Humulus lupulus, Rosmarinum officinalis, Hypericum perforum* and *Valeriana officinalis.*

Though traditional and alternative medicine and its practitioners exist in Europe, it is not officially recognized and is punishable under the law in France, Italy, Spain and other countries, while it is unregulated in UK. This requires provisions in pharmacopoeias to include herbal drugs. Allopathic medicine is practiced predominantly in developed countries, and herbal drugs are categorized as food supplements and are not reimbursed by the social security system.

**ANCIENT SYSTEM OF MEDICINE IN INDIA**

**The two Ancient Medicinal systems**

**Ancients system of medicine types**

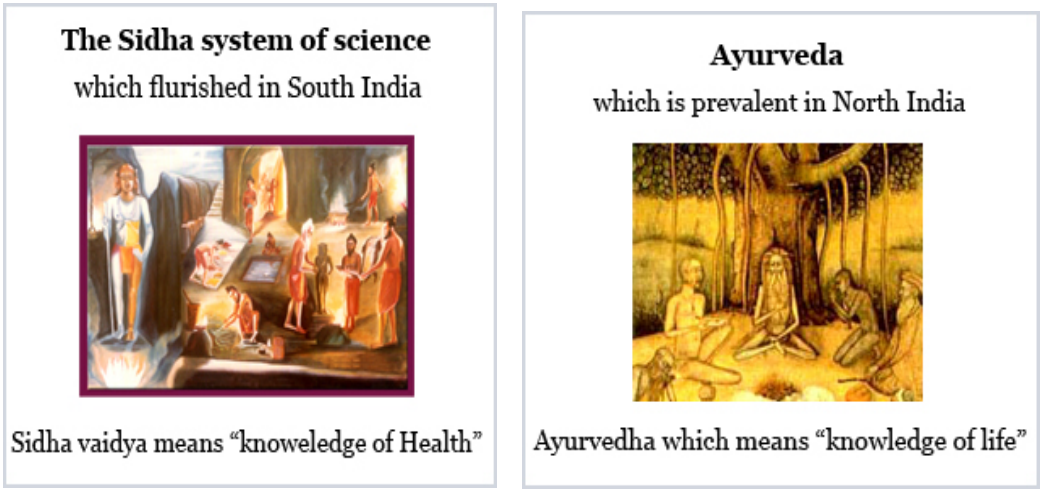


**Figure-2**

**Sidha System of Medicine**

Sidha system developed with the ancient civilizations of Harrappa and Mohenjo-daro, in the Indus River valley, about 6000 - 7000 years ago[42].

It was transplanted into Southern India along with the Dravidian people who migrated to south about 2500 years ago and during this migration, many plants were added into the medicine chest of Siddha treatment.

The Siddha System of medicine is the oldest and it is in vogue from the growing of vegetable kingdom on the Earth. Siddha generally refers to Ashtama Siddhi that is the 8th supernatural power[40]. Those who attained or achieved the above said powers are known as Siddhars. There were 18 important Siddhars in olden days and they developed this system of medicine. Hence, it is called Siddha Medicine. In the Indian History everyone knows that, prior to the Aryan migration, the Dravidians were the first inhabitants of India - of whom the South Indians / Tamilians were the most prominent.

Siddha Vaidya is also the most ancient recorded herbal medical system. Siddha. It is systematic medical system, accurately defined, documented symptomatology and solution for reactions[43]. The well researched remedies make it an ideal one, as a bridge between the modern medicine and other complimentary medical systems.

Siddha Vaidya is very effective; Certain key ingredients like Amber, under some unique process can act instantly as effective as the modern system of Science. It is not true that one has to wait for days and months for a cure under this system. This makes this system unique and superior to other alternative systems. Some varieties of internal medicines, if prepared once can be used for hundred years. Such medicines have long life time and their potency will not be lost at any time, in this period. Siddha Vaidya recognizes eight branches of medicine, the same as western medicine except Geriatrics, the study of diseases of the aged. Instead Rasayana or rejuvenation therapy is given. The aim of their

therapy is to maintain the youth of the individual along with long life. This is attainable through cleanses of the body (Pancha Karma) done seasonally and rejuvenation treatments which activate the body's ability to rebuild and renew itself. According to Siddha Vaidya and Ayurveda, it is the perfect digestion and assimilation of our food, together with the regular and efficient evacuation of wastes that is essential for health and beauty. Any food, no matter how perfect, that is improperly digested, forms toxic wastes called 'Ama'. Ama clogs the system, impedes digestion, blocks vital channels and clouds the mind[43]. Siddha Vaidya is very effective in managing chronic diseases and degenerative conditions and holds many promises for the Health and Wellness of humanity. Healthy, functional and productive longevity is a promise from Siddha Vaidya. Another contribution is its wealth of knowledge regarding prophylaxis of heart diseases.

**Nano technology**

A nanometer is one billionth of a meter, the width of six hydrogen atoms, or about 100,000th the size of a single grain of sand. Nano technology involves the manufacture and manipulation of molecules from 1-100 nanometers in size. A technological revolution that will irreversibly alter the way people live and work. It was said that by 2010, half of all drugs will be made with nano technology[40]. Today only we are talking about nano technology. Siddha System for centuries has been practicing the division of minerals into atomic weights. Atomic weight of hydrogen is 1.0079 atoms. Which is 1/6th the width of one nanometer.

It is this science and who ever practicing to this scale are the makers of superior medicines . For medicines to be very effective the inorganic substances are to be brought to their atomic form and ionic form. Today, We have on hand just a few of these works. Siddha system is all about not only treating illness but promoting youthful maintenance and rebuilding of the body.

Rejuvenation does not necessarily mean restoring the Old to Youth; for it may simply mean maintenance of youth without reaching the old age; and if youth

is maintained perpetually, it becomes Immortality. So Rejuvenation is a means for prolonging life.

Immortality being one of the most consoling and comforting dogma of religion should be reserved for aspiration of Man's faith. It is proved that certain single cell creatures do not yield to natural death. Dr. Alexis Carrel, the celebrated biologist in his article on "tissue culture" has proved beyond doubt that it has been possible to keep some animal tissue almost alive and growing indefinitely in a suitable organic media[43]. He called them as "immortal". Cultures should, at frequent intervals, be transplanted to fresh media or else growth ceases and the cells begin to die poisoned by the accumulation of their debris and other used up stuffs. Similarly for the cells to be at best state, the cells of our body as well should be brought up in an environment, free from the contamination of the waste poisonous by-products in the assimilating process.

Therefore a high degree of constancy is to be maintained in the internal environment for the existence of life and health in the body, by keeping up the equilibrium of acid and alkaline balance in the body, eliminating the waste products regularly.

**Ayurveda System of Medicine**

The word Ayurveda is made up of two words Ayu and Veda where in Ayu stands for life and Veda means science or knowledge. Thus Ayurveda means the Science of Life, Ayurveda looks not only into the physical aspect of life but it also goes deep into its humane aspect also.

It is postulated that as long as the components of soul and mind are intact, the biological forms of the material elements present in our body keep on functioning actively and keep us alive[36].

Around 1500 B.C., the science of Ayurveda advanced systematically and was divided in eight main clinical branches.

- ✓ Internal Medicine (Kaya chikitsa)
- ✓ Surgery, Eye, ENT (Shalya chikitsa)
- ✓ Obstetrics, Gynaecology, Paediatrics (Kaumar bhritya)
- ✓ Pharmacology (Dravya-guna)
- ✓ Personal care, Hygiene (Swastha-vritta)
- ✓ Alchemy (Rasa shastra, Bhaishajya kalpana)
- ✓ Prevention of diseases and improving immunity and rejuvenation (rasayana)
- ✓ Aphrodisiacs and improving health of progeny (Vajikaranam)

As per Ayurveda, There are three basic constituents of human body:

- ✓ Dosha –depict the functional aspects
- ✓ Dhatu –depict the structural composition
- ✓ Mala –are end products of metabolism

**Dosha;** Good health is achieved through ***Doshas*** which are considered vital components (catalysts) responsible for natural catabolic, anabolic and other physiological functions. When their levels are balanced, they maintain good health[38].

There are of three types of ***Doshas***

1.Vata –movements, catabolic process
Equated to nervous / endocrine system
2.Pitta –enzymatic functions, body heat
Equated to digestive system
3.Kapha –anabolic processes, defence mechanism
Equated to lymphatic system

**Dhatu;** They are seven in number:

1.Rasa –Fluids of body
2.Rakta –Blood
3.Mamsa –Muscular tissue
4.Meda –Adipose tissue, fat

5.Asthi –Bones, skeletal tissue

6.Majja –Bone marrow

7.Shukra –Semen / sperm, egg

**Malas**

Distinct for each Dhatu

Rasa –Kapha (phlegm)

Rakta –Pitta (bile)

Mamsa –Vasa (fat)

Meda –Sweda (perspiration)

Asthi-Nakha, smashru, danta

Majja –Akshi, nasa, snehaShukra –Smashru / oja

**Ayurveda in India**

Up to 80% of people in India use either Ayurveda or other traditional medicines.

In 1970, the Indian Medical Central Council Act which aims to standardize qualifications for ayurveda and provide accredited institutions for its study and research was passed by the Parliament of India[37]. In India, over 100 colleges offer degrees in traditional ayurvedic medicine. The Indian government supports research and teaching in ayurveda through many channels at both the national and state levels, and helps institutionalize traditional medicine so that it can be studied in major towns and cities. The state-sponsored Central Council for Research in Ayurvedic Sciences (CCRAS) has been set up to research the subject. To fight biopiracy and unethical patents, the Government of India, in 2001, set up the Traditional Knowledge Digital Library as repository of 1200 formulations of various systems of Indian medicine, such as ayurveda, unani and siddha. The library also has 50 traditional ayurveda books digitized and available online.

Central Council of Indian Medicine (CCIM) a statutory body established in 1971, under Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), Ministry of Health and Family Welfare, Government of India, monitors higher education in ayurveda. Many clinics in urban and rural areas are run by professionals who qualify from these institutes.

Ayurveda stresses the use of plant-based medicines and treatments. Hundreds of plant-based medicines are employed, including cardamom and cinnamon. Some animal products may also be used, for example milk, bones, and gallstones. In addition, fats are used both for consumption and for external use. Minerals, including sulfur, arsenic, lead, copper sulfate and gold are also consumed as prescribed. This practice of adding minerals to herbal medicine is known as *rasa shastra*[39].

In some cases, alcohol was used as a narcotic for the patient undergoing an operation. The advent of Islam introduced opium as a narcotic. Both oil and tar were used to stop bleeding. Traumatic bleeding was said to be stopped by four different methods ligation of the blood vessel; cauterisation by heat; using different herbal or animal preparations locally which could facilitate clotting; and different medical preparations which could constrict the bleeding or oozing vessels. Various oils could be used in a number of ways, including regular consumption as a part of food, anointing, smearing, head massage, and prescribed application to infected areas.

**Herbal Treatments for Medically Challengable Disease**

The use of herbal supplements has increased dramatically over the past 30 years. Herbal supplements are classified as dietary supplements by the U.S. Dietary Supplement Health and Education Act (DSHEA) of 1994. That means herbal supplements -- unlike prescription drugs -- can be sold without being tested to prove that they are safe and effective. However, herbal supplements must be made according to good manufacturing practices.

The most commonly used herbal supplements in the U.S. include echinacea (*Echinacea purpurea* and related species), St. John's wort (*Hypericum perforatum*), ginkgo (*Ginkgo biloba)*, garlic (*Allium sativum*), saw palmetto (*Serenoa repens*), ginseng (*Panax ginseng,* or Asian ginseng; and *Panax quinquefolius,* or American ginseng), goldenseal (*Hydrastis canadensis*), valerian (*Valeriana officinalis*), chamomile (*Matricaria recutita*), feverfew (*Tanacetum parthenium*), ginger (*Zingiber officinale*), evening primrose (*Oenothera biennis*), and milk thistle (*Silybum marianum*).

Often, herbs may be used together because the combination is more effective and may have fewer side effects. Health care providers must take many factors into account when recommending herbs, including the species and variety of the plant, the plant's habitat, how it was stored and processed, and whether or not there are contaminants (including heavy metals and pesticides).

Herbal medicine is used to treat many conditions, such as asthma, eczema, premenstrual syndrome, rheumatoid arthritis, migraine, menopausal symptoms, chronic fatigue, and irritable bowel syndrome, among others. Herbal supplements are best taken under the guidance of a trained health care provider. Be sure to consult with your doctor or pharmacist before taking any herbs. Some common herbs and their uses are discussed below.

- Ginkgo (*Ginkgo biloba*) has been used in traditional medicine to treat circulatory disorders and enhance memory. Although not all studies agree, ginkgo may be especially effective in treating dementia (including Alzheimer's disease) and intermittent claudication (poor circulation in the legs). It also shows promise for enhancing memory in older adults. Laboratory studies have shown that ginkgo improves blood circulation by dilating blood vessels and reducing the stickiness of blood platelets. By the same token, this means ginkgo may also increase the effect of some blood-thinning medications, including aspirin. People taking blood-thinning medications should ask their doctor before using ginkgo.

- Kava kava (*Piper methysticum*) is said to elevate mood, well-being, and contentment, and produce a feeling of relaxation. Several studies have found that kava may be useful in the treatment of anxiety, insomnia, and related nervous disorders. However, there is serious concern that kava may cause liver damage. It's not clear whether the kava itself caused liver damage in a few people or whether it was taking kava in combination with other drugs or herbs. It's also not clear whether kava is dangerous at previously recommended doses, or only at higher doses. Some countries have taken kava off the market. It remains available in the United States, but the Food and Drug Administration (FDA) issued a consumer advisory in March of 2002 regarding the "rare" but potential risk of liver failure associated with kava-containing products.
- Saw palmetto (*Serenoa repens)* is used by more than 2 million men in the United States for the treatment of benign prostatic hyperplasia (BPH), a non-cancerous enlargement of the prostate gland. A number of studies suggest that the herb is effective for treating symptoms, including too-frequent urination, having trouble starting or maintaining urination, and needing to urinate during the night. But a well-conducted study published in the February 9, 2006 edition of the *New England Journal of Medicine* found that saw palmetto was no better than placebo in relieving the signs and symptoms of BPH.
- St. John's wort (*Hypericum perforatum*) is well known for its antidepressant effects. In general, most studies have shown that St. John's wort may be an effective treatment for mild to moderate depression, and has fewer side effects than most other prescription antidepressants. But the herb interacts with a wide variety of medications, including birth control pills, so it is important to take it only under the guidance of a health care provider.
- Valerian (*Valeriana officinalis)* is a popular alternative to commonly prescribed medications for sleep problems because it is considered to be both safe and gentle. Some studies bear this out, although not all have

found valerian to be effective. Unlike many prescription sleeping pills, valerian may have fewer side effects such as morning drowsiness.

- Echinacea preparations (from *Echinacea purpurea* and other *Echinacea* species) may improve the body's natural immunity. Echinacea is one of the most commonly used herbal products, but studies are mixed as to whether it can help prevent or treat colds. A meta-analysis of 14 clinical studies examining the effect of echinacea on the incidence and duration of the common cold found that echinacea supplements decreased the odds of getting a cold by 58%. It also shortened the duration of a cold by 1.4 days.

## 1.1 TARGETED DRUG DELIVERY SYSTEM

Novel drug delivery technologies(NDDS) are revolutionizing the drug delivery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this regard NDDS have benefits includes improves therapy by increase the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved site specific delivery to reduce unwanted adverse effects[52].

**Targeted drug delivery**, sometimes called **smart drug delivery** is a method of delivering medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others[45].

**The goal of a targeted drug delivery system**

- ✓ Drug release prolongation
- ✓ Localized drug effect
- ✓ Target and have a protected drug interaction with the diseased tissue.

The conventional drug delivery system is the absorption of the drug across a biological membrane, whereas the targeted release system is when the drug is released in a dosage form.

Targeted drug delivery systems have been developed to optimize regenerative techniques. The system is based on a method that delivers a certain amount of a therapeutic agent for a prolonged period of time to a targeted diseased area within the body. This helps maintain the required plasma and tissue drug levels in the body[49]. Therefore, avoiding any damage to the healthy tissue via the drug. The drug delivery system is highly integrated and requires various disciplines, such as chemists, biologist and engineers, to join forces to optimize this system.

A successful targeted drug delivery system comprises three elements:

- Orientation cumulation
- Control over drug release
- Non-toxic and biodegradable

**Important Therapeutic Features of Tdds Include**;

1. Sufficient targeting effectiveness, circulation time, and safety (ie., lack of Systemic and local adverse effects)
2. Precise subcellular localization of drugs targeted to endothelial cells
3. adequate amplitude, kinetics, and duration of effects

Recent research has focused on the design of phytosome and polymer based carrier for drug targeting.

Drug carrier are substances that serve as mechanisms to improve the delivery of drugs. Drug carriers are used in sundry drug delivery systems[44].

**The advantages to the targeted release system**

- ✓ Reduction in the frequency of the dosages taken by the patient
- ✓ More uniform effect of the drug
- ✓ Reduction of drug side effects
- ✓ Reduced fluctuation in circulating drug levels

The disadvantage of the system is high cost which makes productivity more difficult and the reduced ability to adjust the dosages.

**Functions of Carrier Drug Delivery System**

1. Optimization of a drugs pharmacokinetics in the bloodstream and protection of drugs against inactivation while maintaining their biological and chemical properties and premature activity and route to the target[47].
2. Fine control of drug release kinetics
3. Providing a template for multivalent affinity findings sites enhancing effectiveness of anchoring on the target cells
4. Modulation of subcellular delivery of drugs. Nanocarriers are broadly defined as submicron structures that can be loaded with drugs

**Controllable Parameters**

Controllable parameters of carriers that define their utility for drug delivery include structural materials, plasticity, morphology, size, shape, permeability, and biodegradability[51].

Carriers which are able to biodegrade include

- Liposome
- **Phytosome**
- Niosome
- Nanoparticle
- Microspheres made of the biodegradable polymer poly(lactic co- glycolic) acid

  Albumin microspheres

  Synthetic polymers (soluble)

  Protein DNA complex
- Resealed Erythrocytes

## PHYTOSOME FOR TARGETING DRUGS

Phytosome one of the novel colloidal drug delivery system that holds great promise for reaching the goal as well as site specific drug release from the vesicles.

Thus phytosome are formulated with natural phytoconstituent for targeting. Today, versatility of particulate technologies enables tailoring of the phytosome-based drug delivery system with the consideration of target, desired pharmacokinetic profile and route of administration[16].

The important technological advantage of phytosome used as a drug carrier are high stability, high carrier capacity, feasibility of incorporation of phytoconstituent and feasibility of routes of administration.

These properties of phytosome enable improvement of drug bioavailability and reduction of the dosing frequency and may resolve the problem of non adherence to prescribed therapy[18].

### Skin as a Targeting Site

Skin is a major target organ for allergic reactions to small molecular weight compounds. Drug allergic reactions may be life-threatening such as in the case of anaphylactic reactions or bullous drug reactions and occur in about 5% of all hospitalized patients. Allergic contact dermatitis has an enormous influence on the social life of the patient because it is the most frequent reason for occupational skin diseases and the treatment and prevention of this disease cost approximately euro 3 billion per year in Germany. The different proposed pathophysiological pathways leading to a drug eruption are discussed in this paper. All major enzymes which are involved in the metabolism of xenobiotic were shown to be present in skin.

## 1.2 Physiology of Skin

**Physiological structures of skin**

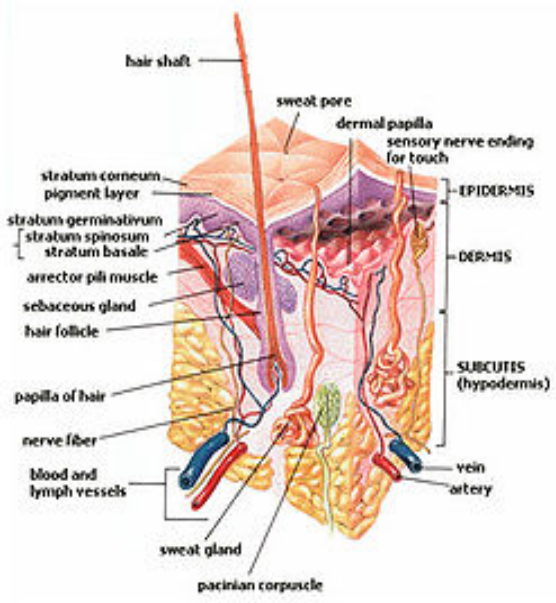


**Figure-3**

The **human skin** is the outer covering of the body. In humans, it is the largest organ of the integumentary system. The skin has multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs. Human skin is similar to that of most other mammals, except that it is not protected by a pelt. Though nearly all human skin is covered with hair follicles, it appears hairless. There are two general types of skin, hairy and glabrous skin. The adjective **cutaneous** literally means "of the skin" (from Latin *cutis*, skin).

Because it interfaces with the environment, skin plays a key role in protecting (the body) against pathogens and excessive water loss. Its other functions are insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates. Severely damaged skin will try to heal by forming scar tissue. This is often discolored and depigmented.

In humans, skin pigmentation varies among populations, and skin type can range from dry to oily. Such skin variety provides a rich and diverse habitat for bacteria which number roughly at 1000 species from 19 phyla.

**Skin** is composed of three primary layers:

- the *epidermis*, which provides waterproofing and serves as a barrier to infection;
- the *dermis*, which serves as a location for the appendages of skin; and
- the *hypodermis (subcutaneous adipose layer)*.

**Epidermis**, "epi" coming from the Greek meaning "over" or "upon", is the outermost layer of the skin. It forms the waterproof, protective wrap over the body's surface and is made up of stratified squamous epithelium with an underlying basal lamina.

The epidermis contains no blood vessels, and cells in the deepest layers are nourished by diffusion from blood capillaries extending to the upper layers of the dermis. The main type of cells which make up the epidermis are Merkel cells, keratinocytes, with melanocytes and Langerhans cells also present. The epidermis can be further subdivided into the following *strata* (beginning with the outermost layer): corneum, lucidum (only in palms of hands and bottoms of feet), granulosum, spinosum, basale. Cells are formed through mitosis at the basale layer. The daughter cells (see cell division) move up the strata changing shape and composition as they die due to isolation from their blood source. The cytoplasm is released and the protein keratin is inserted. They eventually reach the corneum and slough off (desquamation). This process is called *keratinization* and takes place within about 27 days. This keratinized layer of skin is responsible for keeping water in the body and keeping other harmful chemicals and pathogens out, making skin a natural barrier to infection.

The epidermis contains no blood vessels, and is nourished by diffusion from the dermis. The main type of cells which make up the epidermis are

keratinocytes, melanocytes, Langerhan cells and Merkels cells. The epidermis helps the skin to regulate body temperature.

Epidermis is divided into several layers where cells are formed through mitosis at the innermost layers. They move up the strata changing shape and composition as they differentiate and become filled with keratin. They eventually reach the top layer called *stratum corneum* and are sloughed off, or desquamated. This process is called *keratinization* and takes place within weeks. The outermost layer of the epidermis consists of 25 to 30 layers of dead cells.

Epidermis is divided into the following 5 sublayers or strata:

- ✓ Stratum corneum
- ✓ Stratum lucidum
- ✓ Stratum granulosum
- ✓ Stratum spinosum
- ✓ Stratum germinativum

**Dermis**

The **dermis** is the layer of skin beneath the epidermis that consists of connective tissue and cushions the body from stress and strain. The dermis is tightly connected to the epidermis by a basement membrane. It also harbors many Mechanoreceptor/nerve endings that provide the sense of touch and heat. It contains the hair follicles, sweat glands, sebaceous glands, apocrin glands, lymphatic vessels and blood vessels. The blood vessels in the dermis provide nourishment and waste removal from its own cells as well as from the Stratum basale of the epidermis.

The dermis is structurally divided into two areas: a superficial area adjacent to the epidermis, called the *papillary region*, and a deep thicker area known as the *reticular region*.

***Papillary region***

The papillary region is composed of loose areolar connective tissue. It is named for its fingerlike projections called *papillae*, that extend toward the epidermis. The papillae provide the dermis with a "bumpy" surface that interdigitates with the epidermis, strengthening the connection between the two layers of skin.

In the palms, fingers, soles, and toes, the influence of the papillae projecting into the epidermis forms contours in the skin's surface. These are called *friction ridges*, because they help the hand or foot to grasp by increasing friction. Friction ridges occur in patterns that are genetically and epigenetically determined and are therefore unique to the individual, making it possible to use fingerprints or footprints as a means of identification.

***Reticular region***

The reticular region lies deep in the papillary region and is usually much thicker. It is composed of dense irregular connective tissue, and receives its name from the dense concentration of collagenous, elastic, and reticular fibers that weave throughout it. These protein fibers give the dermis its properties of strength, extensibility, and elasticity.

Also located within the reticular region are the roots of the hair, sebaceous glands, swea glands, receptors, nails, and blood vessels.

Tattoo ink is held in the dermis. Stretch marks from pregnancy are also located in the dermis.

**Hypodermis**

The hypodermis is not part of the skin, and lies below the dermis. Its purpose is to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves. It consists of loose connective tissue and elastin.

The main cell types are fibroblasts, macrophages and adiposities (the hypodermis contains 50% of body fat). Fat serves as padding and insulation for the body.

Microorganisms like *Staphylococcus epidermidis* colonize the skin surface. The density of skin flora depends on region of the skin. The disinfected skin surface gets recolonized from bacteria residing in the deeper areas of the hair follicle, gut and urogenital openings.

**Drug Transport Across Skin**

The stratum corneum is the outermost desquamating 'horny' layer of skin, comprising about 15-20 rows of flat, partially desiccated, dead, keratinized epidermal cells. Depending upon the region of the body, the thickness of this layer ranges from 10-20 μm, with the thickest layer on the palms of the hands and soles of the feet. Of the various skin layers, it is the stratum corneum that is the rate-limiting barrier to percutaneous drug transport. In fact, the stratum corneum is a remarkably more formidable barrier to drug transport than the epithelial barriers of gastrointestinal, nasal, buccal, vaginal, or rectal delivery routes.

Transport of hydrophilic or charged molecules is especially difficult attributable to the lipid-rich nature of the stratum corneum and its low water content; this layer is composed of about 40% lipids, 40% protein, and only 20% water. Transport of lipophilic drug molecules is facilitated by their dissolution into intercellular lipids around the cells of the stratum corneum. Absorption of hydrophilic molecules into skin can occur through 'pores' or openings of the hair follicles and sebaceous glands, but the relative surface area of these openings is barely 1% of the total skin surface. This small surface area limits the amount of drug absorption.

Percutaneous absorption of drug molecules is of particular importance in the case of transdermal drug delivery systems because the drug has to be absorbed to an adequate extent and rate to achieve and maintain uniform, systemic,

therapeutic levels throughout the duration of use. In general, once drug molecules cross the stratum corneal barrier, passage into deeper dermal layers and systemic uptake occurs relatively quickly and easily.

Generally, drug absorption into the skin occurs by passive diffusion. The rate of drug transport across the stratum corneum follows Fick's Law of Diffusion (see box below). In other words, the rate of drug transport depends not only on its aqueous solubility, but is also directly proportional to its oil/water partition coefficient, its concentration in the formulation vehicle, and the surface area of the skin to which it is exposed; it is inversely proportional to the thickness of the stratum corneum. The stratum corneum is thickest in the plantar (soles) and palmar regions and thinnest in the postauricular, axillary, and scalp regions of the body. An understanding of the transport behavior of drugs is vital for designing an effective topical or transdermal product, as well as reasonably predicting and comparing drug behavior in various formulations. The latter is of practical importance to the pharmacist who is required to suggest one or more effective drug products out of the many commercial formulations available or to counsel patients on proper use and handling of topical and transdermal products.

**Fick's Law of Diffusion** as applied to drug transport across stratum corneum

$$\frac{dM}{dt} = \frac{D\Delta CK}{h}$$

where,

| | | |
|---|---|---|
| dM/dt | = | is the steady-state flux across stratum corneum |
| D | = | is the diffusion coefficient or diffusivity of drug molecules |
| ΔC | = | is the drug concentration gradient across the stratum corneum |
| K | = | is the partition coefficient of the drug between skin and formulation medium, and |
| h | = | is the thickness of the stratum corneum |

## 1.3 PHYTOSOME - A REVIEW

Over the past century, scientific technology has established the compositions, biological activities, and health-promoting benefits of numerous botanical products. Water-soluble phytoconstituents like flavonoids, tannins, terpenoids, etc. are poorly absorbed either due to their large molecular size, which cannot be absorbed by passive diffusion, or due to their poor lipid solubility, severely limiting their ability to pass across the lipid-rich (outer membranes of the enterocytes of the small intestine) biological membranes, resulting in poor bioavailability.

An ideal drug delivery system is the one that delivers the drug at a rate dictated by the need of the body, over the period of treatment, and channels the active entity solely to the site of action. For this purpose, a number of carriers have been used, like immunoglobins, erythrocytes, reverse micelles, phytosomes, pharmacosomes, etc. Phytosomes have improved pharmacokinetic and pharmacological parameters that result in the treatment of acute and chronic liver disease of toxic metabolic or infective origin or of a degenerative nature. These are also widely used in anti-inflammatory activity and pharmaceutical and cosmetic compositions.

Phytosomes are novel drug delivery system containing hydrophilic bioactive phytoconstituents of herbs surround and bound by phospholipids.

**Structure of Phytosome**

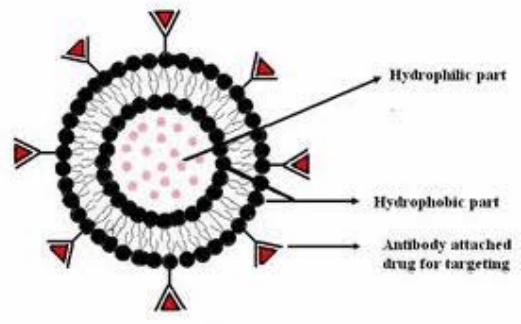


**Figure-4**

Development of phytosomes is at the budding stages in India and abroad. It has a lot of potential in the field of medicine, pharmaceuticals and cosmetics.

The technology has improved pharmacokinetics and pharmacological parameters.

It is found to be safe and efficacious, which in result can advantageously be used in the treatment of various diseases of human beings and animals.

These drug-phospholipid complexes can be formulated in the form of solution, suspension, emulsion, syrup, lotion, gel, cream, aqueous microdispersion, pill, capsule, powder, granules and chewable tablet

For good bioavailability, natural products must have a good balance between hydrophilicity (for dissolving into the gastro-intestinal fluids) and lipophilicity (to cross lipidic biomembranes).

Many phytoconstituents like polyphenolics have good water solubility, but are, nevertheless poorly absorbed because of their large size, incompatible with a process of passive diffusion and/or their poor miscibility with oils and other lipids.

As a result, the ability of flavonoids to cross the lipid-rich outer membrane of small intestine enterocytes is severely limited.

Water-soluble phytoconstituents (mainly polyphenolics) can be converted into a lipid-compatible molecular complex known as Phytosomes.

A Phytosome is generally more bioavailable than simple herbal extract due to its enhanced capacity to cross the lipid-rich biomembranes and reach circulation.

Phospholipids are small lipid molecules where glycerol is bonded to two fatty acids, while the third hydroxyl, normally one of the two primary methylenes, bears a phosphate group bound to a biogenic amino or to an amino acid thus making Phytosomes different from liposomes.

Phospholipids from soy, mainly phosphatidylcholine, are lipophilic agents, and readily complex polyphenolics. In this context, phosphatidylcholine, the major molecular building block of cell membranes and a compound miscible in both water and in oil/lipid environments, is well absorbed orally, and has the potential to act as a chaperon for polyphenolics, shuttling them through biological membranes

**Preparation of Phytosome**

Phytosomes are prepared by reacting from 3-2 moles but preferably with one mole of natural or synthetic phospholipids, with one mole of component like flavolignans, either alone or in the natural mixture in aprotic solvent such as dioxane or acetone.

The phytosome complex can be then isolated by precipitation with non solvent such as aliphatic hydrocarbons or lyophilization or by spray drying. In the complex formation of phytosomes the ratio between these two moieties is in the range from 0.5- 2.0 moles. After the organic solvent was removed under vacuum condition, phospholipid complex was formed.

In the phytosome preparations, phospholipids are selected from the group consisting of soy lecithin, from bovine or swine brain or dermis, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine in which acyl group may be same or different and mostly derived from palmitic, stearic, oleic and linoleic acid.

Selection of flavonoids are done from the group consisting of quercetin, kaempferol, quercretin-3, rhamnoglucoside, quercetin-3-rhamnoside, hyperoside, vitexine, diosmine, 3- rhamnoside, (+) catechin, (-) epicatechin, apigenin-7-glucoside, luteolin, luteolinglucoside, ginkgonetine, isoginkgonetine and bilobetine. [20] Phytosomes are different than liposomes in the way it incorporates the water soluble drug to form the complex.

A liposome is formed by mixing a water soluble substance with phosphatidylcholine in definite ratio under specific conditions. Here, no chemical bond is formed; the phosphatidylcholine molecules surround the water soluble substance.

In contrast, the phytosome process the phosphatidylcholine and the plant components actually form a 1:1 or a 2:1 molecular complex depending on the substance(s) complexed, involving chemical bonds.

Fundamental differences are that in liposomes, the active principles are dissolved in the central part of the cavity, with no possibility of molecular interaction between the surrounding lipid and a hydrophilic substance. On the other hand the phytosome complex can somewhat be compared to an integral part of the lipid membrane, where the polar functionalities of the lipophilic guest interact via hydrogen bonds with the polar head of a phospholipids (i.e. phosphate and ammonium groups), forming a unique pattern which can be characterized by Spectroscopy.

This difference results in phytosome being much better absorbed than liposomes showing better bioavailability.

Phytosomes are also superior to liposomes in skin care products while the liposome is an aggregate of many phospholipids molecules that can enclose other phyto active molecules but without specifically bonding to them. Liposomes are

touted delivery vehicles, but for dietary supplements their promise has not been fulfilled.

But for phytosome products numerous studies prove they are markedly better absorbed and have substantially greater clinical efficacy. Companies have successfully applied this technology to a number of standardized flavonoids preparations.

Some liposomal drugs complex operate in the presence of the water or buffer solution where as phytosomes operate with the solvent having a reduced dielectric constant. Starting material of component like flavonoids is insoluble in chloroform, ethyl ether or benzene. They become extremely soluble in these solvents after forming phytosomes. This chemical and physical property change is due to the formation of a true stable complex.

**Difference between Phytosome and Liposome**

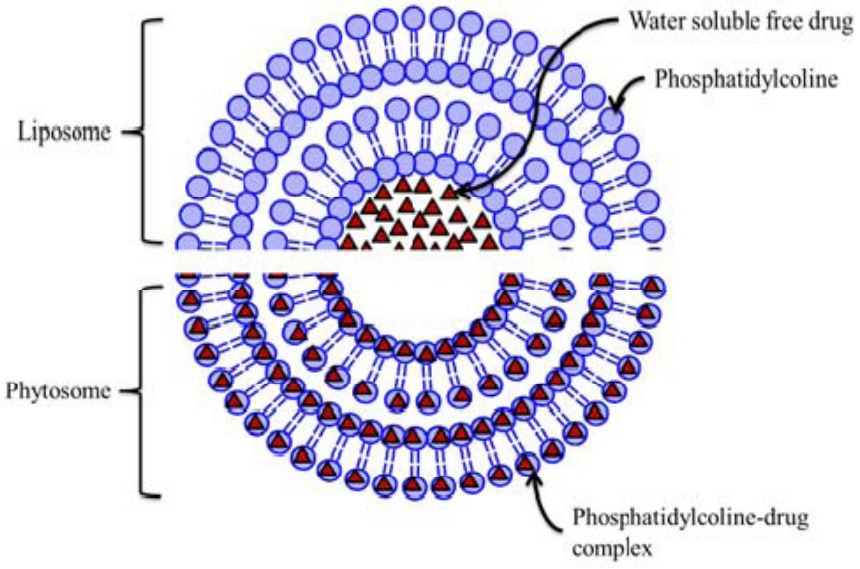


**Figure-5**

**Characterization of Phytosome**

The behavior of phytosomes in both physical and biological system is governed by the factors such as physical size, membrane permeability; percentage entrapped solutes, chemical composition, quantity and purity of the starting materials.

Therefore, the phytosomes are characterized for physical attributes like shape, size, distribution, percentage drug capture entrapped volume, percentage drug released and chemical composition.

Complexation and molecular interactions between phytoconstituents and phosphatidylcholine in solution have been studied by $^{1}$H-NMR ,$^{13}$C-NMR, $^{31}$PNMR, [26] as well as by IR spectroscopy .

Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) are other techniques employed for the detection and measurement of thermal effects such as fusion, solid–solid transitions, glass transitions, loss of solvent, and decomposition to characterize a solid phytosome.

Further NMR data available on the marketed phytosomes also indicates that the signals of the fatty chain are almost unchanged. Such evidences inferred that the two long aliphatic chains are wrapped around the active principle, producing a lipophilic envelope, which envelope the polar head of the phospholipids and the herbal extract.

**Physical and Chemical Properties of Phytosomes**

They are lipophilic substances with a definite melting point, freely soluble in non polar and aprotic solvents in which the hydrophilic moiety is not.

They are moderately soluble in fats and insoluble in water. When treated with water, they assume a micelle shape, forming structures which resemble liposome.

In these complexes, the polar head of the phospholipid is involved while the fatty acid moieties retain a high degree of mobility conferring marked lipophilia at the new molecule.

In the 1H-NMR spectrum, the signals of the complexed substances undergo a strong broadening so as they can no more be evidenced in the spectra.

In the 13C-NMR spectrum, the signals of the complexed substances as well as those of the choline and glycerin portion of the phospholipid can no more be recorded .

The phosphorous nucleus itself undergoes a band broadening which indicates that it is involed in complex formation. In both the 1H-NMR and 13C-NMR spectra, only the lipid chain signals appear, even showing some immobilization.

The kind of signals proves the interaction between polar head and active sites of the complex whereas the lipid chains are not involved since they are free to rotate and give complex its lipophilic character.

**Advantages of Phytosome**

Phytosomes have the following advantages

1) Phytosome are better bioavailable botanical extracts, dramatically enhance bioavailability due to their complex with phospholipids and delivers faster and improved absorption in intestinal tract.
2) They enhance the absorption of lipid insoluble polar phytoconstituents through oral as well as topical route showing better bioavailability with significantly better therapeutic benefit.
3) Dose requirement can be minimized as the bioavailability is increased.
4) Phosphatidylcholine used in preparation of phytosomes besides acting as a carrier also acts as a hepatoprotective substance showing the synergistic effect when hepatoprotective substances like flavanoids are employed to form complex.
5) Phytosome are widely used in cosmetics due to there more skin penetration and high lipid profile.
6) Phytosomes show better stability profile owing to the chemical bonds formed between phosphatidylcholine molecule and phytoconstituents.

## 1.4 WART OVERVIEW

A **wart** is generally a small, rough growth, typically on a human's hands or feet but often other locations, that can resemble a cauliflower or a solid blister.

They are caused by a viral infection, specifically by one of the many types of *human papillomavirus*. There are as many as 10 varieties of warts, the most common considered to be mostly harmless. It is possible to get warts from others; they are contagious and usually enter the body in an area of broken skin. They typically disappear after a few months but can last for years and can recur.

### Types

A range of types of wart have been identified, varying in shape and site affected, as well as the type of *human papillomavirus* involved. These include:

- Common wart (*Verruca vulgaris*), a raised wart with roughened surface, most common on hands, but can grow anywhere on the body;
- Flat wart (*Verruca plana*), a small, smooth flattened wart, flesh-coloured, which can occur in large numbers; most common on the face, neck, hands, wrists and knees;
- Filiform or digitate wart, a thread- or finger-like wart, most common on the face, especially near the eyelids and lips;
- Genital wart (venereal wart, *Condyloma acuminatum*, *Verruca acuminata*), a wart that occurs on the genitalia.
- Mosaic wart, a group of tightly clustered plantar-type warts, commonly on the hands or soles of the feet;
- Periungual wart, a cauliflower-like cluster of warts that occurs around the nails.
- Plantar wart (verruca, *Verruca plantaris*), a hard sometimes painful lump, often with multiple black specks in the center; usually only found on pressure points on the soles of the feet;

**CAUSES**

Warts are caused by the *Human papilloma virus* (HPV). There are about 130 known types of *human papilloma viruses*. HPV infects the squamous epithelium, usually of the skin or genitals, but each HPV type is typically only able to infect a few specific areas on the body. Many HPV types can produce a benign growth, often called a "wart" or "papilloma", in the area they infect. Many of the more common HPV and wart types are as follows:

- Common warts - HPV types 2 and 4 (most common); also types 1, 3, 26, 29, and 57 and others
- Cancers and Genital dysplasia - "high-risk" HPV types are associated with cancers, (cervical cancer and can also cause some vulvar, vaginal, penile, anal and some oropharyngeal cancers. "low-risk" types are associated with warts or other conditions)
- High-risk: 16, 18 (cause the most cervical cancer); also 58, 33, 45, 31, 52, 35, 39, 59, and others
- Plantar warts (myrmecia) - HPV type 1 (most common); also types 2, 3, 4, 27, 28, and 58 and others
- Anogenital warts (condylomata acuminata or venereal warts) - HPV types 6 and 11 (most common); also types 42, 44 and others
- Low-risk: 6, 11 (most common); also 13, 44, 40, 43, 42, 54, 61, 72, 81, 89, and others
- Flat warts - HPV types 3, 10, and 28
- Butcher's warts - HPV type 7
- Heck's disease (Focal epithelial hyperplasia) - HPV types 13 and 32.

**Pathophysiology**

Common warts have a characteristic appearance under the microscope. They have thickening of the stratum corneum (hyperkeratosis), thickening of the stratum spinosum (acanthosis), thickening of the stratum granulosum, rete ridge elongation, and large blood vessels at the dermoepidermal junction.

**Prevention**

Gardasil is a HPV vaccine aimed at preventing cervical cancers and genital warts. Gardasil is designed to prevent infection with HPV types 16, 18, 6, and 11. HPV types 16 and 18 currently cause about 70% of cervical cancer cases and also cause some vulvar, vaginal, penile and anal cancers. HPV types 6 and 11 are responsible for 90% of documented cases of genital warts. Unfortunately the HPV vaccines do not currently prevent the virus strain responsible for verrucas (plantar warts)

**Treatment**

There are many treatments and procedures associated with wart removal. One review of 52 clinical trials of various cutaneous wart treatments concluded that topical treatments containing salicylic acid were the best supported, with an average cure rate of 75%, compared with 48% for the placebo in six placebo-controlled trials including a total of 376 participants. The reviewers also concluded that there was little evidence of a significant benefit of cryotherapy over salicylic acid or duct tape.

One complicating factor in the treatment of warts is that the wart may regrow after it has been removed.

Treatments that may be prescribed by a medical professional include

- Application of podophyllum resin paint [podophyllum resin I.P.'66 (20% w/v), benzoin I.P. (10% w/v), aloes I.P. (2% w/v), isopropyl alcohol I.P. to make (100% v/v)]

- Imiquimod, a topical cream that helps the body's immune system fight the wart virus by encouraging interferon production. Approved by the U.S. Food and Drug Administration (FDA) for genital warts. The drug is very expensive
- Cantharidin, a chemical found naturally in many members of the beetle family Meloidae which causes dermal blistering. Either used by itself or compounded with podophyllin Not FDA approved, but available through Canada or select US compounding pharmacies.
- Bleomycin, not USFDA approved. One or two injections used. It can cause necrosis of digits and Raynaud syndrome
- Dinitrochlorobenzene (DNCB), like salicylic acid, this is applied directly to the wart. Studies showed this method was effective with a cure rate of 80%. But DNCB must be used much more cautiously than salicylic acid; the chemical is a known mutagen, able to cause genetic mutations. So a physician must administer DNCB. This drug induces an allergic immune response resulting in inflammation that wards off the wart-causing virus
- Fluorouracil, which inhibits DNA synthesis, is being used as an experimental treatment. It is applied directly to the wart (especially plantar warts) and covered (for example: with tape). This treatment is combined with the use of a pumice stone, but tends to work very slowly
- Salicylic acid can be prescribed by a dermatologist in a higher concentration than that found in over-the-counter products. Examples include a topical solution marketed by Elorac, Inc. under the trade name Durasal

There are several over-the-counter options. The most common ones involve salicylic acid. These products are readily available at drug stores and supermarkets. There are typically two types of products: adhesive pads treated with salicylic acid or a bottle of concentrated salicylic acid solution. Removing a wart with salicylic acid can be done by cleaning the area, applying the acid, and

removing the dead skin with a pumice stone or emery board. It may take up to a year to remove a wart.

Another product available over-the-counter that can aid in wart removal is silver nitrate in the form of a caustic pencil, which is also available at drug stores. In a placebo-controlled study of 70 patients, silver nitrate given over nine days resulted in clearance of all warts in 43% and improvement in warts in 26% one month after treatment compared to 11% and 14%, respectively, in the placebo group. The instructions must be followed to minimize staining of skin and clothing. Occasionally pigmented scars may develop.

Cryosurgery or cryotherapy devices using a dimethyl ether - propane mixture are inexpensive. A disadvantage is that the sponge applicator is too large for small warts, and the temperature achieved is not nearly as low as with liquid nitrogen. Complications include blistering of normal skin if excess freezing is not controlled.

Several randomized, controlled trials have found that zinc sulfate, consumed orally, often reduces or eliminates warts. The zinc sulfate dosage used in medical trials for treatment of warts was between 5 and 10 mg/kg/day. For elemental zinc, a lower dosage of 2.5 mg/kg/day may be appropriate as large amounts of zinc may cause a copper deficiency. Other trials have found that topical zinc sulfate solution or zinc oxide are also effective.

**Procedures**

- Keratolysis, of dead surface skin cells usually using salicylic acid, blistering agents, immune system modifiers ("immunomodulators") or formaldehyde, often with mechanical paring of the wart with a pumice stone, blade etc.,
- Electrodesiccation

- Cryosurgery, which involves freezing the wart (generally with liquid nitrogen), creating a blister between the wart and epidermal layer, after which the wart and surrounding dead skin falls off by itself. An average of 3 to 4 treatments are required for warts of thin skin. Warts on calloused skin like plantar warts might take dozens or more treatments.
- Surgical curettage of the wart
- Laser treatment - often with a pulse dye laser or carbon dioxide ($CO_2$) laser. Pulse dye lasers (wavelength 582 nm) work by selective absorption by blood cells (specifically haemoglobin). $CO_2$ lasers work by selective absorption by water molecules. Pulse dye lasers are less destructive and more likely to heal without scarring. $CO_2$ laser works by vaporizing and destroying tissue and skin. Laser treatments can be painful, expensive (though covered by many insurances) and can cause little scarring when used appropriately. $CO_2$ lasers will require local anaesthetic. Pulse dye laser treatment does not need conscious sedation nor local anesthetic. It takes 2 to 4 treatments but can be many more for extreme cases. Typically, 10-14 days are required between treatments. Preventative measures are important
- Infrared coagulator - an intense source of infrared light in a small beam like a laser. This works essentially on the same principle as laser treatment. It is less expensive. Like the laser, it can cause blistering pain and scarring
- Duct tape occlusion therapy involves placing a piece of duct tape over the wart. The evidence as to whether or not it is effective is poor. Thus it is not recommended as routine treatment.

**Folk Remedies**

A variety of traditional folk remedies and rituals claim to be able to remove warts. In *The Adventures of Tom Sawyer*, Mark Twain has his characters discuss a variety of such remedies. Tom Sawyer proposes "spunk-water" (or "stump-water", the water collecting in the hollow of a tree stump) as a remedy for warts on the hand.

One remedy for warts involves rubbing the wart with a potato, which is then buried; when the "buried potato dries up, the wart will be cured"

Another remedy similar to Twain's is reported from Northern Ireland, where water from a specific well on Rathlin Island is credited with the power to cure warts

# CHAPTER - 2

# LITERATURE REVIEW OF THUJA OCCIDENTALIS

**Kim JY *et.al.,* 2011**, *Thuja occidentalis* (TO) has been a recognized herbal medicine across Northeast Asian countries for thousands of years and used for the treatment of various inflammatory diseases through as yet undefined mechanisms. In this study, he concluded that that the anti-inflammatory effects of this plant are mediated to suppress mitogen-activated protein kinases (MAPKs) and nuclear factor-κB (NF-κB) activation in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells.

**Huaping *et.al.,* 2010,** The essential oils from leaves, twigs and stems of large trees and shrub-like trees of Thuja sutchuenensis were extracted by hydrodistillation and supercritical fluid extraction, and analyzed by GC and GC-MS. The essential oil composition differed significantly among the three organs, as well as between large trees and shrub-like trees. Furthermore, consistent with the eastern Asia-North American disjunct distribution of the genus, many differences in the essential oil composition between *T. sutchuenensis* and other Thuja species were apparent. The essential oils exhibited a certain degree of antifungal activity against six strains of human pathogenic fungi.

**Lee YJ *et.al.,* 2010**, Studied *in vitro* screening of 600 medicinal plant extracts, an aqueous extract of *Thuja occidentalis* (ATO) was found to exhibit antiinflammatory activity in human umbilical vein endothelial cells (HUVEC). In the current study, the anti inflammatory activity of ATO and possible mechanisms for this were investigated in HUVEC. Pre incubation with ATO (20□μg/ml) suppressed tumor necrosis factor-α (TNF-α)-induced expression of adhesion molecules including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin at both the protein and mRNA levels. ATO also inhibited U937 monocyte adhesion to HUVEC stimulated by TNF-α. In addition, ATO attenuated TNF-α-induced p65 NF-κB translocation into

the nucleus and phosphorylation of IκB-α. Furthermore, ATO significantly inhibited TNF-α-induced intracellular reactive oxygen species (ROS) production. Overall, the present data suggest that ATO can suppress TNF-α-induced vascular inflammatory processes, possibly via inhibition of ROS and NF-κB activation in HUVEC.

**B.K.Ojeswi *et.al.*, 2010,** *In vivo* experiment has been conducted to observe the preventive role of *Thuja occidentalis* Linn (leaves) against 7, 12 dimethylbenz(a)anthracene (DMBA)-induced mammary cancer. Ethyl acetate (EtOAc) and methanolic (MeOH) extracts in two doses (5 and 10 mg/kg body weight) of the plant were tested for DMBA-induced Indian Cancer Research Centre (ICRC) mice mammary carcinoma in terms of tumor weight, volume, life span, histological variation and oxidative stress against the reference drug doxorubicin using standard animal protocol. EtOAc extract (10 mg/kg body weight) of the plant exhibits reduction of tumor weight (39%), tumor volume (50%), reduced glutathione (GSH) (83%) and malignant cells compared to cancerous control group while the increase in body weight and life span in comparison with cancerous control and doxorubicin-treated group. EtOAc extract being most potent extract has been subjected to detailed chromatographic separation. The most potent chromatographic fraction exhibits the presence of flavonoidal unit. Structural elucidation of bioactive principle is in progress. It is inferred that the plant *T. occidentalis* (leaves) possess significant potential for phytopreventive bioefficacy against DMBA-induced mammary carcinogenesis

**Dimitroula Tsiri *et.al.*, 2009,** identified essential oils of four varieties of Thuja species cultivated in Poland - *T. occidentalis*, *T. occidentalis*, *T. plicata* - were investigated by GC and GC-MS. Thirty-one compounds were identified from *T. occidentalis* 'globosa', representing 96.92% of the total oil; twenty-seven from *T. occidentalis* 'aurea' (94.34%); thirty-one from *T.plicata* (94.75%); and thirty compounds from *T. plicata* 'gracialis' (96.36%). The main constituents in all samples were the monoterpene ketones alpha- and beta-thujone, fenchone and sabinene, as well as the diterpenes beyerene and rimuene. The chemosystematic

value of the total ketone content of all samples (which varied from 54.30-69.18%) has been discussed and investigated. The constituents, beyerene and the mixture of alpha- and beta-thujone, were isolated from the oils and tested against six Gram-positive and -negative bacteria and three pathogenic fungi. The oils of the two *T. plicata* species exhibited significant antimicrobial activity, while the mixture of alpha- and beta-thujone showed very strong activity as well.

**Choo SJ *et.al.,* 2009,** Demonstrated free radical scavenging and antielastase activity of Flavanoids from the extract of *Thuja occidentalis*. And he concluded that The free radical scavenging and human neutrophil elastase (HNE) inhibitory activities were evaluated for the isolated compounds by using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.

**Sunila ES *et.al.,* 2006**, Demonstrated *Thuja occidentalis* extract on the inhibition of lung metastasis induced by B16F-10 melanoma cells was studied in C57BL/6 mice. The extract was administered by three different modalities. A remarkable reduction in tumor-nodule formation was shown by simultaneous (74.4%) and prophylactic (71.5%) mode of administration. The effect was comparatively low in drug administration after tumor development (60.2%). Increased lung collagen hydroxyproline (21.13 microg/mg protein) in the metastasized lungs of control animals compared with normal animals (0.98 microg/mg protein) was significantly reduced in Thuja-treated animals

**Sunila ES *et.al.,* 2005**, was carried the research on *Thuja occidentalis* against damage induced by gamma radiation in mice. Whole-body exposure of Swiss albino mice to gamma-rays (6 Gy) reduced the total white blood cell count to 1900 cells/mm(3) on the third day, which was elevated to 2050 cells/mm(3) by the administration of alcoholic extract of *T.occidentalis* (5 mg/dose/animal, intraperitoneally). Six animals from each group were killed after 2, 7, and 11 days of irradiation to detect the bone marrow cellularity and radiation-induced toxicity. The number of bone marrow cells and alpha-esterase positive cells in control animals after 11 days was reduced to 12.2 x 10(6) cells/femur and 693.5/4000

cells, respectively. In *T occidentalis*-treated animals, bone marrow cellularity was increased to 16.9 x 10(6) cells/femur and alpha-esterase positive cells were 940/4000 cells, a nearly normal level. Alcoholic extract of *T.occidentalis* reduced the elevated levels of GPT and alkaline phosphatase in liver and serum after irradiation.

**Girish gupta *et.al.*, 2002**, Antimycotic Potential of *Thuja occidentalis* against Curvularia-lunata causing Phaeoyphomycosis was studied. In Human, Various potencies of a homoeopathic drug, *Thuja-occidentalis*-Q 30, 200, 1M, 10M and 50M and Ketoconazole (a synthetic dioxolane imidazole compound-a broad spectrum antifungal) as a positive control were evaluated by following Poison food method. Finally he concluded that, *Thuja* 30 and 200 was found highly effective even more than positive control and vehicle.

**Offergeld R *et.al.*,1998**,Studied the antiviral & immune stimulatory activity of Thuja occidentalis was shown to be an inducer of the CD4+ fraction of the human peripheral blood T-cell subset (1,2). Furthermore, it could be demonstrated that TPSg is a potent inhibitor of the expression of HIV-1-specific antigens and of the HIV-1-specific reverse transcriptase (3). This report deals with the cytokine pattern induced by TPSg in human peripheral blood lymphocyte (PBL) and purified monocyte/macrophage cultures. In addition, a further characterization of the CD4+ T-cell fraction stimulated by TPSg was performed by FACS analysis. TPSg is induces IL-1 beta, IL-2, IL-3, IL-6, gamma-IFN, G-CSF, GM-CSF, and TNF-beta production in PBL cultures; and IL-1 beta and IL-6 in monocyte/macrophage cultures. Enzyme-linked immunosorbent assays (ELISAs) demonstrated that no IL-4 was produced by PBL cultures under TPSg influence.

**Belal naser *et.al.*, 1995**, Demonstrated antiviral and immunopharmacological potential, such as stimulatory and co-stimulatory effects on cytokine and antibody production and activation of macrophages and other immunocompetent cells. And he concluded thuja occidentalis having effective antiviral and immunostimulatory effects.

## 2.1 LITERATURE REVIEW OF WART

**A Shakoor *et.al.*, 2012,** Demonstrated the Effects of *Thuja Occidentalis* as an alternative remedy in the treatment of Papillomatosis in Cattle. Papillomatosis is a condition in farm animals and is treated by surgical excision or application of certain caustic agents. Contrary to it, two cattle with teat warts were treated by an alternative method by using the *Thuja occidentalis* (thuja-30, a homeopathic medicine) 10 drops twice a day for a span of 3 weeks and lead to complete recovery.

**Anca Gaston *et.al,* 2012,** Salicylic acid and cryotherapy are the most frequent treatments for common warts, but can be painful and cause scarring, and have high failure and recrudescence rates. Topical vitamin A has been shown to be a successful treatment of common warts. He demonstrated with healthy, physically-active 30 old female with a 9 year history of common warts on the back of the right hand. The warts resisted treatment with salicylic acid, apple cider vinegar and an over-the-counter blend of essential oils marketed for the treatment of warts. Daily topical application of natural vitamin A derived from fish liver oil (25,000 IU) led to replacement of all the warts with normal skin. Most of the smaller warts had been replaced by 70 days. A large wart on the middle knuckle required 6 months of vitamin A treatment to resolve completely.

**Yu-guo Pu *et.al.*, 2009,** Interferon has been widely used in the treatment of genital warts for its immunomodulatory, antiproliferative and antiviral properties. Currently, no evidence that interferon improves the complete response rate or reduces the recurrence rate of genital warts has been generally provided. He demonstrated with involving 1445 people were included. Among them, 7 studies demonstrated the complete response rate of locally-used interferon as compared to placebo for treating genital warts. Based on meta-analysis, the rate of Complete response of the two interventions differed significantly (locally-used interferon:44.4%; placebo:16.1%).and finally he concluded that,Interferon tends to be a fairly well-tolerated form of therapy. According to different routes of

administration, locally-used interferon appears to be much more effective than both systemically-used interferon and placebo in either improving the complete response rate or reducing the recurrence rate for the treatment of genital warts.

**Allice Rosenberg *et.al.,* 2008**, Carried the research on genital wart by treating them with cryotherapy. And he demonstrated that the Untreated anogenital warts can spread and form large clusters that are bothersome, painful, and embarrassing to the patient as well as being a significant health risk. Approximately 10% of HIV positive patients have anogenital warts. He concluded that, the anogenital warts are successfully treated with cryotherapy.

**U.R. Hengge *et,al.,* 2008**, Imiquimod, a novel topical immune response modifier, has been successfully used for the treatment of external anogenital warts. Demonstrated the safety, tolerance and efficacy of imiquimod for the treatment of common cutaneous warts and mollusca that were resistant to previous therapeutic interventions. Imiquimod 5% cream was self-applied by the patients to the warts or mollusca once daily for 5 days per week and left in place overnight. Patient-applied 5% imiquimod cream holds promise as an effective treatment of common warts and mollusca in a difficult-to-treat patient population.

**Rachel Wenner. MD *et.al.,* 2007,** Evaluated the efficacy of duct tape occlusion therapy for the treatment of common warts in adults. A total of 90 immunocompetent adult volunteers with at least 1 wart measuring 2 to 15 mm were enrolled between October 1, 2004, and July 31, 2005. Eighty patients completed the study. Finally they concluded that the no statistically significant difference between duct tape and moleskin for the treatment of warts in an adult population.

**Katherine M. Stone *et.al.,* 2004,** The treatment of genital warts remains frustrating since it is often painful, expensive, and unsuccessful. Moreover, little is known about the infectivity and natural history of exophytic genital warts or subclinical genital infection with *human papillomavirus*. The traditional goals of

therapy for sexually transmitted diseases—eradication of infection, elimination of symptoms, prevention of long-term sequelae, and interruption of transmission—are currently not attainable for or applicable to genital warts. The medical literature from January 1988 to August 1993 was reviewed for recent studies on the treatment of exophytic warts. The following treatments were included in the reviewed studies: podofilox (which was recently approved by the Food and Drug Administration), podophyllin, cryotherapy, topical 5-fluorouracil, intralesional interferon, systemic interferon, and laser surgery.

**Stephen tyring *et.al.*, 1998,** Published the article about the treatment of wart by the application of o.5% podophyllox topical cream and demonstrated the effective inhibition of *pappiloma virus.*

**MD Malison *et.al.*, 1994,** Demonstrated, that autogenous vaccine is an effective form of immunotherapy for condyloma acuminatum, a double-blind cross-over study was carried out on 34 patients, in which autogenous wart vaccine was compared with a placebo vaccine identically prepared from each patient's own normal skin. Finally they concluded that autogenous vaccine are more effective treatment for warts.

## 2.2 LITERATURE REVIEW OF PHYTOSOME

**Varsha Kashaw *et.al.*, 2011,** Liver an imperative organ has a crucial in the metabolism of xenobiotics that causes it to succumb to numerous hepatic diseases. Synthetic drugs exploited in the treatment of liver diseases are incompetent and may sometimes lead to serious side-effects. In this context, herbal therapy has emerged as a proficient approach with good values in treating hepatic diseases. Medicinal plants may serve as a vital source of potentially useful new compounds for the development of effective therapy to combat a variety of liver problems. Many herbs have been proven to be effectual as hepatoprotective agents while many more are claimed to be hepatoprotective but lack any such scientific evidence to support such claims. Developing a satisfactory herbal therapy to treat severe liver diseases requires systematic investigation of properties such as antiviral action (Hepatitis B, Hepatitis C), anti-hepatotoxicity (antioxidants), stimulation of liver regenerationand choleretic activity. Formulation of herbal medicines with standards of safety and efficacy can revitalize treatment of liver disorders. The focus of this review is to elucidate the concept of hepatotoxicity, including its causes, pathogenesis and prevention. And he concluded that the scope of herbal plants as well as novel delivery systems like liposomes and phytosomes for the treatment of hepatotoxicity and liver related disorders.

**Rahila ahmad pathan *et.al.*, 2011**, Prepared a gymnemic acid-phospholipid complex (GPC) formulation in attempt to enhance the lipophilicity and to characterize the new developed formulation. Gymnemic acid (GA), due to its water soluble complex and large structure, restrict permeation across biological membrane causes poor bioavailability by oral route. To improve the bioavailability and prolong its duration in body system, its phospholipid complexes were prepared by a simple and reproducible method. GPC was formulated by mechanical dispersion method. Using aqueous ethanol as a reaction medium, GA and phospholipids were resolved into the medium, after the solvent was removed under vacuum condition, GPC was formed. In this new formulation, complex formation was confirmed by carrying out by FTIR, $^{1}$H-NMR, XRD,

DSC, and microscopical studies. Solubility and in vitro studies were carried out to ascertain the solubility and dissolution pattern of free and complexed GA. Content of GA in GPC was found 96.27% (w/w). FTIR, $^{1}$H NMR, DSC and XRD data confirmed the formation of phospholipid complex. n-Octanol solubility was altered for free GA and GPC, 8.6 µg to 137 µg/ml. Unlike the free GA this showed a burst and rapid drug release, GPC showed sustained release at the end of 120 minutes of dissolution study in phosphate buffer. Microscopy also shows the entrapment of GA in the lipid core showing complex structure, which is supported by change in surface morphology of GA when complexed with phospholipid. Hence he concluded that, findings demonstrate that complexing GA with phospholipids results in better n-Octanol solubility, enhance lipophilicity, and altered physicochemical profile compared to free GA which can lead to improved biological effects.

**Sunitha manthena *et.al.*, 2010,** Studied the Phytoconstituents, despite having excellent bioactivity *in vitro*, demonstrate less or no *in vivo* actions due to their poor lipid solubility or improper molecular size or both, resulting in poor absorption and poor bioavailability. Lipid solubility and molecular size are the major limiting factors for molecules to pass the biological membrane and to be absorbed systematically following oral or topical administration. Some phytoconstituents are destroyed in the gastric environment when taken orally. The term "phyto" means plant, while "some" means cell-like. Therefore, phytosome is a "phytophospholipid complex" resembling a small cell. Phytosomes are produced by a patented process whereby standardized plant extracts or their constituents are bound to phospholipids, mainly phosphatidylcholine, producing a lipid-compatible molecular complex. Phytosomes exhibit a better pharmacokinetic and pharmacodynamic profile than conventional herbal extracts. And concluded that, The phytosome technology markedly enhances the bioavailability of phytomedicine and has effectively enhanced the bioavailability of many popular herbal extracts, including *Milk thistle, Ginkgo biloba, Grape seed, Green tea,*

*Hawthorn, Ginseng* etc., and can be developed for various therapeutic uses or dietary supplements

**Javed *et.al.*, 2010,** The present article dwells in reviewing critically the patents published mainly on the new emerging trends and techniques for increasing the bioavailability of silymarin, the polyphenolic fraction obtained from the seeds of Silybum marianum. The use of this herb for treating various ailments like hepatitis, cirrhosis, jaundice, mushroom and toxin poisoning is well known. But the potential use of this herbal drug is limited due to the poor absorption and poor bioavailability after oral administration. The belief that the natural medicines are much safer than synthetic drugs, has gained popularity in recent years and led to tremendous growth of phytopharmaceutical usage and thus the need of improving the bioavailability by various means like complexation, derivatization, solubilization, targeted delivery, controlled delivery and many other miscellaneous techniques. They concluded that phyto phospholipid complex are showing better pharmacokinetic profile than other bioavailability enhancement technique for phytoconstituent

**J. Naga Sowjanya *et.al.*, 2010,** Carried out the research in natural medicinal plants. Several plant extracts and phytoconstituents, despite having excellent bioactivity in vitro demonstrate less or no in vivo actions due to their poor lipid solubility or improper molecular size or both, resulting poor absorption and poor bioavailability. So, much work has been directed towards the development of new concept in herbal delivery system i.e., "Phytosomes" which are better absorbed, utilized and as a result produce better results than conventional herbal extracts owing to the presence of phosphatidylcholine which likely pushes the phytoconstituent through the intestinal epithelial cell outer membrane, subsequently accessing the bloodstream. And he concluded that the Phytosomes have improved pharmacokinetic and pharmacological parameter which in result can advantageously be used in the treatment of the acute and chronic liver disease of toxic metabolic or infective origin or of degenerative nature. It can also be used

in anti-inflammatory activity as well as in pharmaceutical and cosmetic compositions.

**Rajendra Awasthi *et.al.*,2010,** Done the research on phytosomal preparation are compared with conventional herbal preparations about their bioavailability and pharmacokinetic and pharmacodynamic properties. Thorough study of literature different phytosome products has demonstrated significant therapeutic or health promoting properties when compared with the conventional plant extracts. Phytosomes can be developed for different therapeutic purposes like hepatoprotective, cardiovascular, liver diseases.

**Sapna malviya *et.al.*, 2009**, Phytosome is usually a flavonoids molecule linked with phosphatidylcholine molecule. A bond is formed between the two molecules, creating a hybrid molecule. This highly lipid-miscible hybrid bond is better suited to merge into the lipid phase of the enterocyte's outer cell membrane and from there into the cell, finally reaching the blood. Phosphatidylcholine is not merely a passive "carrier" for the bioactive flavonoids of the phytosomes, but is itself a bioactive nutrient with documented clinical efficacy for diseases, including alcoholic hepatic steatosis, drug-induced liver damage, hepatitis, Alzheimer's, brain aging, skin diseases, cardiovascular diseases such as congestive heart failure and it also act as an effective antioxidant. And finally he concluded that phytosome are showing better pharmacokinetic profile for various diseased conditions.

**Mukesh S. Sikarwar *et.al.*, 2008,** Formulated the Marsupsin–phospholipid complex (M–P Complex) in attempt to increase the bioavailability of marsupsin and to characterize this new formulation along with its evaluation. Marsupsin–phospholipid complex was formulated by mechanical dispersion method. In this new formulation, complex formation was confirmed by carrying out transmission electron microscopy (TEM), IR, 1H-NMR and RP-HPLC analysis. TEM showed M–P Complex diameter range of 0.05–0.5 μm. The entrapment efficiency of M–P Complex was found to be 44%. In vitro release study revealed its first order

release profile. Mean blood serum concentration vs time curve of marsupsin was of first order after oral administration of M–P Complex in albino rabbits which clearly showed remarkably increased bioavailability of M–P Complex than standardized marsupsin. The average value of Cmax and Tmax of M–P Complex were found to be 3.02 mg/ml and 10.2 h, respectively. Hence the findings demonstrate that complexing marsupsin with phospholipids results in better oral bioavailability and improved biological response than free form of standardized marsupsin.

**Amit gupta *et.al.*, 2007,** Bioavailability of lipophilic drugs when administered orally as solid dosage form is significantly low. There are usually several factors responsible for this, but a particularly widespread problem is poor absorption due to slow and/or incomplete drug dissolution in the lumen of the gastrointestinal tract. The phytosomes according to the invention have a marked lipophilic character and unexpectedly improve oral/topical absorption of complexed and consequently show improved specific activity in the various pharmacological tests made by experimental methods. The phytosome process intensifies herbal compounds by improving absorption, increasing bioavailability and enhancing delivery to the tissues. Finally concluded that phytosome technology enables cost effective delivery and synergistic benefits form the phospholipid nutraceuticals intrinsic to life.

## NEED FOR THE PRESENT STUDY

Wart is a solid outgrowth on the epidermis Caused by viral infection, specifically by one of the many types of *Human papilloma virus*. There are 10 varieties of warts, They typically disappear after a few months but can last for years and can reoccur.

Wart treatment should be harmless to normal skin surface, painless, inexpensive and should not form any scar on the treated surface. Also the drug should act on the affected cells only with avoidance of recurrence.

Currently available wart treatment procedures and wart removal procedures are complicated and should not satisfy the above requirements. The allopathic treatments are more painful and the recurrence of infection is possible.

Based on the above reasons Complementary Alternative System of Treatments are selected to treat Wart effectively with the plant *Thuja occidentalis* extract. The cytotoxic property of *Thuja occidentalis* act on the thickened layer of stratum corneum (hyperkeratosis), thickening of the stratum spinosum (acanthosis) and thickening of the stratum granulosum. It enhances the immune system by stimulating T-lymphocytes and increasing interleukin-2 production. It reduces the size of wart.

Thus, the need for the present study is to encapsulate the *Thuja occidentalis* extract in to Phytosome for effective targeting of wart.

### WHY PHYTOSOME?

The phosphatidyl choline and cholesterol increases the fluidity and permeability of skin surfaces.

Inclusion of cholesterol in the preparation of phytosome has been demonstrated to alter the properties of phytosome and markedly decreasing the efflux of entrapped solid. Since decreasing efflux by even 1/3 would both increase

the peak concentration and the half time in the plasma by 3 fold, greatly enhancing the therapeutic effect of this antiwart treatment.

Phosphatidyl choline has greatly impacted the drug delivery technology. The very first and most important advantage of phospholipid based vesicular system is the compatibility of phospholipids with membrane of human either internal membrane as well as skin (external membrane). For a drug to be absorbed and distributed into organs and tissues and eliminated from the body, it must pass through one or more biological membranes/ barriers at various locations. Such a movement of drug across the membrane is called as drug transport. For the drugs to be delivered to the body should cross the membranous barrier. Either it would be from oral route or topical/transdermal route. Therefore the phospholipid based carrier systems are of considerable interest in this era. A number of drug delivery systems are based entirely on Phosphatidyl choline such as Liposome, Ethosome, Phytosome, Transferosomes, and Nanocochelates.

The targeted delivery of Phytosomal formulation of *Thuja occidentalis* containing the constituent flavonoid is potent, so it is more suitable to deliver by targeted drug delivery.

The active constituents may be accumulated in the skin by this technique.

The Phytosome are formulated by the formation chemical bond between the extract and phosphatidyl choline, thus the phytosome are more stable than liposome.

# PLAN OF WORK

## 1.EXTRACTION AND IDENTIFICATION

- ✓ Collection of plant
- ✓ Authentification of plant
    - Drying and Powdering of Leaves
    - Selection of solvents
    - Extraction by Soxylation
- ✓ Phytochemical Analysis
- ✓ Characterization of Extract
    - Absorption maxima
    - Solubility tests
    - FT- Infrared study
    - Calibration curve

## 3.MATERIALS AND EQUIPMENTS USED

## 4.FABRICATION AND CHARACTERIZATION PHYTOSOMAL DDS

a. Fabrication of Phytosome

- Solvent evaporation technique
- Optimization of various parameters in Phytosomal formulation

b. Compatibility studies

- FT Infra red spectrum

c. Characterization of Phytosome

- Shape – SEM
- Size
- Particle Size Distribution

- Drug Entrapment efficiency
- Drug content analysis

d. *In-vitro* studies

- *In-vitro* diffusion study-Drug release profile

**6. INCORPORATION OF SELECTED FORMULATION into GEL BASE**

a. Physicochemical evaluation

b. *In-vitro* diffusion study of Phytosomal Gel- release profile

c. Comparison of Extract Phytosome, Phytosomal Gel with marketed Thuja Gel

d. Stability testing

e. Animal studies – Skin irritation studies

**7. RESULTS AND DISCUSSION**

**8. SUMMARY AND CONCLUSION**

# MATERIALS AND EQUIPMENTS

## List of Materials used for Phytosomal preparation

| S.No | Materials Used | Manufacturer |
|---|---|---|
| 1 | *Thuja occidentalis* | Local vendors, Trichy. |
| 2 | Cholesterol | Sigma-Aldrich Cheme, Germany. |
| 3 | Phosphatidyl choline | Qualigens fine chemicals, Mumbai. |
| 4 | Methanol | Sigma-Aldrich cheme, Germany. |
| 5 | Chloroform | Himedia, Mumbai. |
| 6 | Pet ether | Ranbaxy, Mumbai. |
| 7 | Ethanol | Ranbaxy, Mumbai. |
| 8 | Sodium alginate | Sigma-Aldrich cheme, Germany. |
| 9 | Distilled water | Ranbaxy, Mumbai. |
| 10 | Triethanolamine | Sigma-Aldrich cheme, Germany. |
| 11 | Triton-X100 | Qualigens, Mumbai. |
| 12 | Potassium dihydrogen ortho phosphate | Ranbaxy , Mumbai. |
| 13 | Disodium hydrogen phosphate | Ranbaxy , Mumbai. |
| 14 | Sodium chloride | Nice chemicals, Cochin. |

**Table-1**

**List of Equipments Used for Phytosomal preparation**

| S.No | Equipments | Manufacturer |
|---|---|---|
| 1 | Rotary Flask Evaporator | Equitron, Medica Instrument Mfg co, Mumbai |
| 2 | Ultra sonicator | Vibronics, Mumbai |
| 3 | UV-Spectrophotometry | Shimadzu 1700, Japan |
| 4 | Electronic Balance | Sartorius, Germany |
| 5 | SEM | Hitachi, Model S-3000 H, Japan |
| 6 | FT-IR | Perkin elmer, USA |
| 7 | Centrifuge | Remi |
| 8 | pH meter | Elico L1120, A.P, India |
| 9 | Magnetic stirrer | Remi, Gujarat. |
| 10 | Environment stability testing chamber | Heco Environment Chamber, Germany |

**Table -2**

# CHAPTER -3

## 3.1 PLANT AND EXCIPIENTS PROFILE

### *THUJA OCCIDENTALIS*

**Figure-1**

**DESCRIPTION**

| | | |
|---|---|---|
| Family | : | Cupressaceae |
| Synonym | : | Tree of Life, Yellow Cedar, American Arborvitae |
| Genus | : | *Thuja* |
| Species | : | *T. occidentalis* |
| Botanical source | : | *Thuja occidentalis* |
| Active constituents | : | Flavonoids, Essential oil (thujone), Volatile oil (0.4- 1%); the main compound is thujone (both alpha-thujone and beta-thujone, up to 65% of the total oil), together with alpha – pinene, camphor, borneol and fenchone. Also the constituens are sesquiterpenes, flavonoids and polysaccharides |

The name "*Thuja"* was given to this group of trees by the Swedish botanist Linnaeus in 1753; it comes from the Greek word *thuo*, which means "to sacrifice," as cedar wood was often burned with animal sacrifices by the ancients to add a pleasing aroma to the fire[1].

*Thuja occidentalis* is native to North America and grows in dense forests in southeastern Canada and the northeastern United States.

American cedar trees grow to a height of about 60 feet (18.2 m), with trunks between 12 and 24 in (31 and 61 cm) in diameter.

They are slow-growing trees, and prefer wet soils. The leaves of the American cedar are bright green, opposite leaves that resemble overlapping scales, and give off a fragrant odor when crushed. The tiny yellow or greenish flowers appear between April and July. American cedar cones are pale green when young but turn a pale reddish-brown color as they mature.

*Thuja* is also the name for a homeopathic remedy made from *Thuja occidentalis*.

**Distribution**

*Thuja occidentalis* is native to Manitoba east throughout the Great Lakes Region and into Québec, Vermont, New Hampshire, Maine, Prince Edward Island, New Brunswick, and Nova Scotia. Isolated populations exist to the south in Massachusetts, Connecticut, Ohio, Kentucky, Tennessee, North Carolina, Pennsylvania, Maryland, Virginia, and West Virginia.

**Genaral Uses**

- ✓ Cedar trees in general have a long history of use for various herbal remedies and aromatherapy preparations
- ✓ In Western herbal medicine, cedar leaf oil was used as an emmenagogue, abortifacient, vermifuge, diuretic, and digestive aid

- ✓ It was applied externally to relieve the pains of arthritis and rheumatism, to treat external fungal infections of the skin (ringworm and thrush), and to remove anal or genital **warts**
- ✓ Native Americans used cedar leaf preparations to relieve headache and to prevent scurvy
- ✓ Cedar leaves and twigs are in fact rich in vitamin C , and it was their effectiveness in preventing or treating scurvy that led to the tree's being called arbor vitae or **tree of life**
- ✓ In addition, recent research has shown that extracts prepared from either *Thuja occidentalis* or *Thuja plicata* do in fact have antiviral, anti-inflammatory, and antibacterial properties
- ✓ A group of German researchers reported in 2002 that an extract prepared from cedar leaf, alcohol, and water inhibits the reproduction of influenza virus type A[2]
- ✓ Another group of Japanese researchers reported in 2003 that several compounds isolated from the stem bark of Japanese cedar appear to have significant antitumor activity
- ✓ In traditional Chinese medicine, the leaves and stems of *Thuja orientalis* are used to treat nervous disorders, insomnia, and heart palpitations, as well as to stop hemorrhages and bring down fevers
- ✓ Traditional Chinese physicians also make a preparation of fresh cedar leaves steeped for seven days in a 60% alcohol solution to promote hair growth. The mixture is rubbed on the bald spots three times daily
- ✓ The homeopathic preparation known as *Thuja* is made from the leaves of *Thuja occidentalis*, and is given to treat soft or bleeding warts on the chin, genitals, or anus
- ✓ The most widely used homeopathic *materia medica*, or reference book, also recommends *Thuja* for headaches, vertigo, emotional depression and restlessness, pain or itching in the scalp

- ✓ In aromatherapy, *Thuja occidentalis* is used for anxiety, asthma, bronchitis, and head colds. Some aromatherapists also recommend cedar leaf oil for treating acne and dandruff

**Available Preparations**

Most products used in Western medicine and aromatherapy that contain cedar oil are made with oil from the leaves and twigs of *Thuja occidentalis*.

These parts of the tree yield about 1% volatile oil, which is about 65% thujone. The other components include fenchone, borneol, limonene, pinene, camphor, myrcene, a flavonoid known as thujin, and tannin.

The oil is extracted from the leaves by a process of steam distillation, cooled in an indirect contact heat exchanger, filtered and stored in barrels for distribution to wholesalers.

For internal use as a diuretic or expectorant, Western herbalists recommend taking 1/4-teaspoonfull of liquid extract of cedar leaf in a glass of water three to six times a day. Alternately, an infusion can be prepared by adding 1 ounce of fresh cedar leaves to a pint of boiling water. The infusion is taken cold in 1-tebspoonfull doses every three to six hours[3].

Homeopathic preparations of *Thuja* include pills, granules, and liquid dilutions, in potencies ranging from 3X to 50M.

Tinctures of *Thuja* are sold only to homeopathic practitioners, Boiron, a well-known manufacturer of homeopathic remedies, Also offers, *Thuja* ointment for the treatment of external warts

**Interactions**

No interactions between products containing cedar oil and prescription medications have been reported as of early 2004.

**Dose**

Oral dose upto 1.25 mg/kg is harmless in human. which is equal to 85 mg in 150 pound person[54].

Single daily dose 75mg is safe in human.

Topical dosage are much higher. The content of Thuja extract is present in most skin care products is usually less than 1%. Thuja extract contains 2.4% thujone, Thus most skin care products contain 0.024% thujone[5].

## 3.2 CHOLESTEROL

**Structure of Cholesterol**

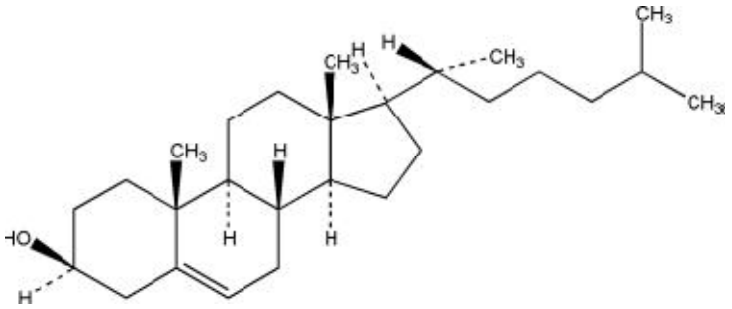


**Figure-2**

The name originates from the Greek word chole-(bile) and stereos(solid), and the chemical suffix – ol for an alcohol, most of the cholesterol in the body is synthesized by the body and some has dietry origin. Cholesterol is more abundant in tissue which either synthesize more or have more abundant densely-packed membranes, for example, the liver, spinal cord and brain. Cholesterol is also considered as sterol (a combination steroid and alcohol)[56].

Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins, complex spherical particles which have an exterior composed mainly of water soluble proteins; fats and cholesterol are carried internally[58].

**Nonproprietary Names**

| | | |
|---|---|---|
| BP | : | Cholesterol |
| JP | : | Cholesterol |
| PhEur | : | Cholesterolum |
| USPNF | : | Cholesterol |

**Synonyms**

Cholesterin

Cholesterolum.

**Chemical Name and CAS Registry Number**

Cholest-5-en-3ßY-ol [57-88-5]

**Functional Category**

- Emollient
- Emulsifying agent

**Excretion;**

Cholesterol is excreted from the liver in bile and reabsorbed from the intestine

**Stability and Storage Conditions**

Cholesterol is stable and should be stored in a well-closed container, protected from light.

**Incompatibilities**

Cholesterol is precipitated by digitonin.

**Applications in Pharmaceutical Formulation or Technology**

Cholesterol is used in cosmetics and topical pharmaceutical formulations at concentrations of 0.3–5.0% w/w as an emulsifying agent. It imparts water-absorbing power to an ointment and has emollient activity[57].

Cholesterol also has a physiological role. It is the major sterol of the higher animals, and it is found in all body tissues, especially in the brain and spinal cord. It is also the main constituent of gallstones[56]

**Properties of cholesterol**

| | |
|---|---|
| IUPAC name | (10R,13R)-10,13,-dimethyl-17-(6-methylheptan-2-yl)-2,3,4,4,7,8,9,11,14,15,16,17,-dodecahydro-1H-cyclopenta[a] phenanthren-3-ol |
| CAS numbers | [57-88-5] |
| Molecular formula | $C_{27}H_{46}O$ |
| Molar mass | 386.654 |
| Appearance | White crystalline powder |
| Melting point | 148°C-150°C |
| Boiling point | 360°C |
| Solubility in water | 0.095 mg/L (30°C) |

**Table -3**

**Sources**

Cholesterol is found in animal fats. All food containing animal fats contains cholesterol; food not containing animal fats either contains no cholesterol or negligible amounts major dietary sources of cholesterol include egg yolks, beef, poultry and shrimp.

## 3.3 LECITHIN

**Structure of Lecithin**

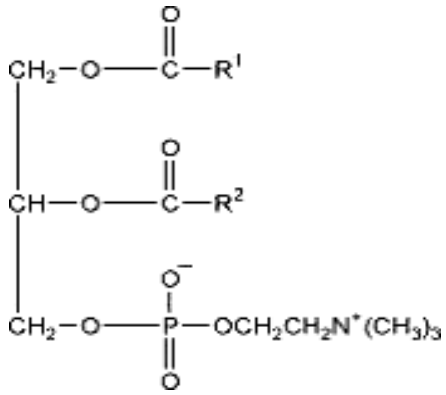

**Figure-3**

Lecithin is a complex mixture of materials; The structure above shows phosphatidylcholine, the principal component of egg lecithin, in its a·-form. In the ß·-form, the phosphorus-containing group and the R2 group exchange positions[55].

**Description**

Lecithins vary greatly in their physical form, from viscous semiliquids to powders, depending upon the free fatty acid content. They may also vary in color from brown to light yellow, depending upon whether they are bleached or unbleached or on the degree of purity. When they are exposed to air, rapid oxidation occurs, also resulting in a dark yellow or brown color.

Lecithins have practically no odor. Those derived from vegetable sources have a bland or nutlike taste, similar to that of soybean oil.

**Nonproprietary Names**

USPNF: Lecithin

**Synonyms**

E322; egg lecithin; *LSC 5050*; *LSC 6040*; mixed soybean phosphatides; ovolecithin; *Phosal 53 MCT*; *Phospholipon 100 H*; soybean lecithin; soybean phospholipids; *Sternpur*; vegetable lecithin.

**Chemical Name and CAS Registry Number**

Lecithin [8002-43-5]

The chemical nomenclature and CAS Registry numbering of lecithin is complex. The commercially available lecithin, used in cosmetics, pharmaceuticals, and food products, is a complex mixture of phospholipids and other materials. However, it may be referred to in some literature sources as 1,2-diacyl-*sn*-glycero-3-phosphocholine (trivial chemical name, phosphatidylcholine). This material is the principal constituent of egg lecithin and has the same CAS registry Number.

**Typical Properties**

**Density:**

0.97 g/cm3 for liquid lecithin;

0.5 g/cm3 for powdered lecithin.

**Iodine number:**

95–100 for liquid lecithin;

82–88 for powdered lecithin.

**Saponification value:** 196

**Solubility**

Lecithins are soluble in aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, mineral oil, and fatty acids. They are practically insoluble in cold

vegetable and animal oils, polar solvents, and water. When mixed with water, however, lecithins hydrate to form emulsions.

**Empirical Formula and Molecular Weight**

The USPNF 23 describes lecithin as a complex mixture of acetone-insoluble phosphatides that consists chiefly of phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl inositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates as separated from a crude vegetable oil source. The composition of lecithin (and hence also its physical properties) varies enormously depending upon the source of the lecithin and the degree of purification. Egg lecithin, for example, contains 69% phosphatidyl choline and 24% phosphatidyl ethanolamine, while soybean lecithin contains 21% phosphatidyl choline, 22% phosphatidyl ethanolamine, and 19% phosphatidyl inositol, along with other components.

**Functional Category**

Emollient

Emulsifying agent

Solubilizing agent.

**Applications in Pharmaceutical Formulation or Technology**

Lecithins are used in a wide variety of pharmaceutical applications, They are also used in cosmetics and food products.

**Concentration of Routes**

| Use | Concentration (%) |
|---|---|
| Aerosol inhalation | 0.1 |
| IM injection | 0.3–2.3 |
| Oral suspensions | 0.25–10.0 |

**Table - 4**

Lecithins are mainly used in pharmaceutical products as dispersing, emulsifying, and stabilizing agents and are included in intramuscular and intravenous injections, parenteral nutrition formulations, and topical products such as creams and ointments.

Lecithins are also used in suppository bases, to reduce the brittleness of suppositories, and have been investigated for their absorption-enhancing properties in an intranasal insulin formulation. Lecithins are also commonly used as a component of enteral and parenteral nutrition formulations.

There is evidence that phosphatidyl choline (a major component of lecithin) is important as a nutritional supplement to fetal and infant development. Furthermore, choline is a required component of FDA-approved infant formula. Other studies have indicated that lecithin can protect against alcohol cirrhosis of the liver, lower serum cholesterol levels, and improve mental and physical performance.

Liposomes in which lecithin is included as a component of the bilayer have been used to encapsulate drug substances; their potential as novel delivery systems has been investigated. This application generally requires purified lecithins combined in specific proportions. Therapeutically, lecithin and derivatives have been used as a pulmonary surfactant in the treatment of neonatal respiratory distress syndrome.

**Stability and Storage Conditions**

Lecithins decompose at extreme pH. They are also hygroscopic and subject to microbial degradation. When heated, lecithins oxidize, darken, and decompose. Temperatures of 160– 180°C will cause degradation within 24 hours.

Fluid or waxy lecithin grades should be stored at room temperature or above; temperatures below 10°C may cause separation. All lecithin grades should be stored in well-closed containers protected from light and oxidation. Purified solid lecithins should be stored in tightly closed containers at subfreezing temperatures.

**Incompatibilities**

Incompatible with esterases owing to hydrolysis.

**Safety**

Lecithin is a component of cell membranes and is therefore consumed as a normal part of the diet. Although excessive consumption may be harmful, it is highly biocompatible and oral doses of up to 80 g daily have been used therapeutically in the treatment of tardive dyskinesia.When used in topical formulations, lecithin is generally regarded as a nonirritant and nonsensitizing material. The Cosmetic Ingredients Review Expert Panel (CIR) has reviewed lecithin and issued a tentative report revising the safe concentration of the material from 1.95% to 15.0% in rinse-off and leave-in products. They note, however, that there are insufficient data to rule on products that are likely to be inhaled.

**Handling Precautions**

Observe normal precautions appropriate to the circumstances and quantity of material handled. Lecithins may be irritant to the eyes; eye protection and gloves are recommended.

## 3.4 SODIUM ALGINATE

### Nonproprietary Names

| | | |
|---|---|---|
| BP | : | Sodium alginate |
| PhEur | : | Natrii alginas |
| USPNF | : | Sodium alginate |

### Synonyms

Algin; alginic acid, sodium salt; E401; *Kelcosol*; *Keltone*; *Protanal*; sodium polymannuronate.

### Chemical Name and CAS Registry Number

Sodium alginate [9005-38-3]

### Empirical Formula and Molecular Weight

Sodium alginate consists chiefly of the sodium salt of alginic acid, which is a mixture of polyuronic acids composed of residues of D-mannuronic acid and L-guluronic acid. The block structure and molecular weight of sodium alginate samples has been investigated

### Functional Category

Stabilizing agent; suspending agent; tablet and capsule disintegrant; tablet binder; viscosity increasing agent.

### Applications in Pharmaceutical Formulation or Technology

Sodium alginate is used in a variety of oral and topical pharmaceutical formulations. In tablet formulations, sodium alginate may be used as both a binder and disintegrant; it has been used as a diluent in capsule formulations. Sodium alginate has also been used in the preparation of sustained-release oral

formulations since it can delay the dissolution of a drug from tablets, capsules, and aqueous suspensions.

In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams and gels and as a stabilizing agent for oil-in-water emulsions. Recently, sodium alginate has been used for the aqueous microencapsulation of drugs, in contrast with the more conventional microencapsulation techniques which use organic solvent systems. It has also been used in the formation of nanoparticles.

The adhesiveness of hydrogels prepared from sodium alginate has been investigated and drug release from oral mucosal adhesive tablets, and buccal gels, based on sodium alginate have been reported. Other novel delivery systems containing sodium alginate include ophthalmic solutions that form a gel *in situ* when administered to the eye; an *in situ* forming gel containing paracetamol for oral administration; and a freeze-dried device intended for the delivery of bone-growth factors Hydrogel systems containing alginates have also been investigated for delivery of proteins and peptides.

Therapeutically, sodium alginate has been used in combination with an $H_2$-receptor antagonist in the management of gastro esophageal reflux, and as a haemostatic agent in surgical dressings. Alginate dressings, used to treat exuding wounds, often contain significant amounts of sodium alginate as this improves the gelling properties. Sponges composed of sodium alginate and chitosan produce a sustained drug release and may be useful as wound dressings or as tissue engineering matrices. Sodium alginate is also used in cosmetics and food products;

**Concentration in formulations**

| Use | Concentration (%) |
|---|---|
| Pastes and creams | 5–10 |
| Stabilizer in emulsions | 1–3 |
| Suspending agent | 1–5 |
| Tablet binder | 1–3 |
| Tablet disintegrant | 2.5–10 |

**Table - 5**

**Description**

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder.

**Typical Properties**

**Acidity/alkalinity**

pH 7.2 for a 1% w/v aqueous solution.

**Solubility**

Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 7. Slowly soluble in water, forming a viscous colloidal solution.

**Viscosity (dynamic)**

Various grades of sodium alginate are commercially available that yield aqueous solutions of varying viscosity. Typically, a 1% w/v aqueous solution, at 20°C, will have a viscosity of 20–400 m Pas (20–400 cP).

Viscosity may vary depending upon concentration, pH, temperature, or the presence of metal ions. Above pH 10, viscosity decreases.

**Stability and Storage Conditions**

Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humidities and a cool temperature.

Aqueous solutions of sodium alginate are most stable at pH 4–10. Below pH 3, alginic acid is precipitated. A 1% w/v aqueous solution of sodium alginate exposed to differing temperatures had a viscosity 60–80% of its original value after storage for 2 years. Solutions should not be stored in metal containers.

Sodium alginate solutions are susceptible on storage to microbial spoilage, which may affect solution viscosity. Solutions are ideally sterilized using ethylene oxide, although filtration using a 0.45 μm filter also has only a slight adverse effect on solution viscosity. Heating sodium alginate solutions to temperatures above 70°C causes depolymerization with a subsequent loss of viscosity. Autoclaving of solutions can cause a decrease in viscosity, which may vary depending upon the nature of any other substances present. Gamma irradiation should not be used to sterilize sodium alginate solutions since this process severely reduces solution viscosity.

Preparations for external use may be preserved by the addition of 0.1% chlorocresol, 0.1% chloroxylenol or parabens. If the medium is acidic, benzoic acid may also be used. The bulk material should be stored in an airtight container in a cool, dry place.

**Incompatibilities**

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenylmercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%. Low concentrations of electrolytes cause an

increase in viscosity but high electrolyte concentrations cause salting-out of sodium alginate; salting-out occurs if more than 4% of sodium chloride is present.

**Safety**

Sodium alginate is widely used in cosmetics, food products, and pharmaceutical formulations, such as tablets and topical products, including wound dressings. It is generally regarded as a nontoxic and nonirritant material, although excessive oral consumption may be harmful. A study in five healthy male volunteers fed a daily intake of 175 mg/kg bodyweight of sodium alginate for 7 days, followed by a daily intake of 200 mg/kg body-weight of sodium alginate for a further 16 days, showed no significant adverse effects.

The WHO has not specified an acceptable daily intake for alginic acid and alginate salts as the levels used in food do not represent a hazard to health.

Inhalation of alginate dust may be irritant and has been associated with industrial-related asthma in workers involved in alginate production. However, it appears that the cases of asthma were linked to exposure to seaweed dust rather than pure alginate dust.

**Handling Precautions**

Observe normal precautions appropriate to the circumstances and quantity of material handled. Sodium alginate may be irritant to the eyes or respiratory system if inhaled as dust; Eye protection, gloves, and a dust respirator are recommended. Sodium alginate should be handled in a well-ventilated environment.

## MATERIALS & METHODS

### Collection of Plant

Fresh leaves of *Thuja occidentalis* were collected from in and around Trichy. The leaves are evergreen, rhomboid-ovate, with a gland on the back, squamose, appressed, and imbricated in 4 rows. The cones are terminal, oblong, and nodding; the scales pointless and 1-seeded; the seeds broadly winged. And it is preserved for further process

### Authentication of Plant

The dried leaves are prepared as herbarium for authentication.

NATIONAL INSTITUTE OF HERBAL SCIENCE
**Plant Anatomy Research Centre : PARC**

Prof.P. Jayaraman, Ph.D
Director
(Retd,Professor,PresidencyCollege
Chennai-5).

# 4, 2nd Street, Sakthi Nagar,
West Tambaram, Chennai-600 045.
Ph:044-22263236, 9444385098
E.mail:herbalparc@yahoo.com

AUTHENTICATION CERTIFICATE

Based upon the organoleptic /macroscopic / ~~microscopic~~ examination of fresh / ~~market~~ sample, it is certified that the specimen given by Mr. R. K. ARTHIK, Dept. of Pharmaceutics, Periyar College of Pharmaceutical Sciences, Trichy-21. is identified as below :

Binomial: Thuja sp.

Family: Cupressaceae - Coniferales.

Synonym(s):

Regional names:

Reg.No of the certificate: PARC/2012/1077

References : Nair, N.C & Henry, A.N. Flora of Tamilnadu, India 1: —— .1983.
Henry, A.N. *et al.* Ibid. —— II: —— .1987.
Ibid. III: p:150.1989.

Date: 19.01.2013.

(Prof.P.JAYARAMAN)
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**Drying and Powdering of Plant**

The fresh leaves were spreaded under shadow place to remove the chlorophyll content in the leaves for seven days. And the colour of the leaves were evaluated in between days. After the shadow drying process the leaves of *Thuja occidentalis* were free from chlorophyll content.

The shadow dried leaves were collected and it was made to grind to get coarse powder for effective soxhlation. To avoid the fine powder formation for good yield value from the extraction. The coarse powder of *Thuja occidentalis* leaves were packed for soxhlation.

The coarse was packed in a thimble, and it was placed over the round bottom flask and above the thimble condenser placed for condensing evaporating vapour in to the thimble.

**Selection of Solvents**

Solvents are playing the major role in extraction process. The extractive solvents otherwise called as menstrum is generally hydroalcoholic (i.e. a mixture of water and alcohol).

Based on the active constituents of *Thuja occidentalis,* solvents like pet ether and ethanol were selected. Pet ether was selected to remove waxy oil constituents from the plant.

The hydroalcoholic solvent ethanol was selected to extract the active constituent of the flavanoid is extracted.

**Extraction by Soxylation**

Extraction may be defined as a process of removing the soluble active principles present in plant tissues from the inactive and insoluble residue by use of selective solvents.

To execute continuous hot extraction, a soxhlet apparatus is used. The soxhlet apparatus is used for the extraction on a small scale. It consist of a flask, a soxhlet extractor and a reflux condenser. The powdered coarse powder of *Thuja occidentalis* placed in a thimble made of filter paper was inserted in to the wide tube of the extractor.

The solvent pet ether was taken in the flask to remove waxy substances from the plant. The solvent was heated and the vapour arises from the flask was get in to the condenser through the side tube and the liquid condensed from the vapours drips in to the thimble. The solvent liquid levels slowly rises and during this period, fat gets extracted of its soluble constituents.

When the level of the liquid reaches the top of the syphon, it gets syphoned in to the flask. The suction effect of the syphoning assists permeation of the solvent through the plant material.

Again, a portion of the solvent from the solution was vapourised leaving the constituents in the flask itself and the process mentioned above is repeated. And the total oil content along with fatty material from the plant was removed by this pet ether extract. And the plant was removed from the thimble and dried in open atmosphere. Pet ether from the plant is removed after the plant was ready to treat with ethanol to execute the active constituents from the plant.

The solvent ethanol was taken in the flask and it was heated. The same process was repeated again and again until all the solutes are extracted. The active constituents containing extractive liquid was processed with rotary film evaporator to remove the solvent ethanol. And the pure extract (i.e. 100% free from solvent) was collected from the flask.

The extract may contain different active constituents, it was identified with the help of infra red studies, chemical tests, solubility test and absorption maxima of extract.

**Soxhlet apparatus**

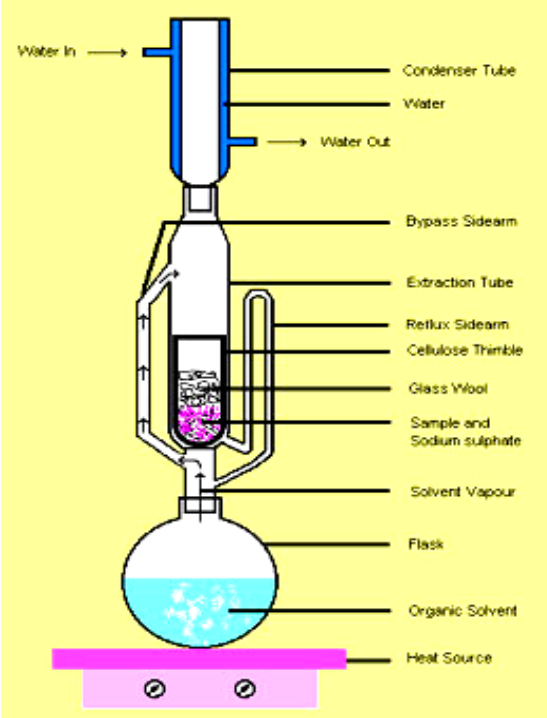


**Figure-4**

**Phytochemical analysis**

The Ethanolic extract of *Thuja occidentalis* is subjected to phytochemical analysis for the determination of phyto compounds like Alkaloids, Corbohydrates, Glycoside, Phenols, Saponins, Phytosterols, Fixed oil and Flavonoids etc.,

**Characterization of Extract**

**Fourier transform infrared (FTIR) spectral analysis**

FT-IR is used to identify the functional groups in the molecule. The drug was mixed with KBr and pellet is formed. Each KBr disk was scanned at 4 mm/s at a resolution of 2 cm over a wave number region of 400 to 4,500 cm−1. The characteristic peaks were recorded. The results are shown in Figure.6 and Table.23

The ethanolic crude extract of *Thuja occidentalis* may contain different active compounds. It was identified with the help of infrared spectrum of the extract.

**Absorption maxima (λ max)**

Drug molecule in solution when exposed to light in the visible/ultraviolet region of the spectrum absorbs light of particular wave length depending on the type of electronic transition associated with the absorption.

The drug solution (1mg/ml) in phosphate buffer pH 6.8 was taken in the cuvette scanned in the range of 230 to 400 nm in a UV spectrophotometer. It exhibits maxima at **361nm**. Therefore, all the further measurements were taken at 361nm

**Reference standard**

*Thuja occidentalis* constituent of flavanoid like quercetin showing the maximum absorption peak at 364 nm in the phosphate buffer 6.8 solution[87].

**Flavonoid absorption maxima**

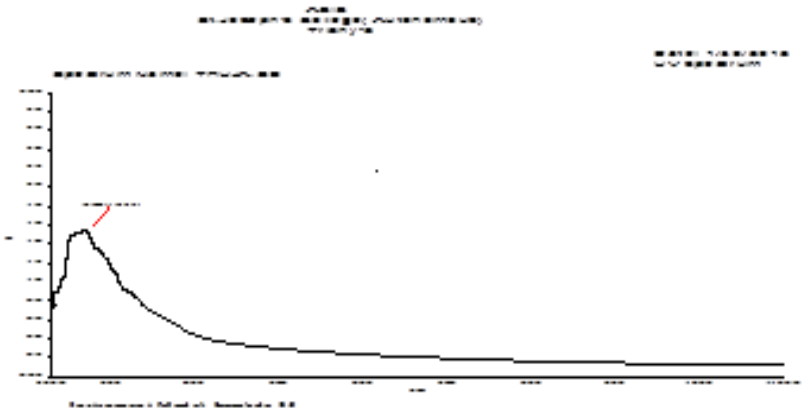

**Figure- 5**

**Solubility Tests**

The spontaneous interaction of two or more substances to form a homogenous molecular dispersion is called solubility. The solubility of crude extract of *Thuja occidentalis* was studied in various solvents. The extract of *Thuja occidentalis* (10mg) was suspended separately in a 10ml of different solvents at room temperaturein tightly closed test tubes and shaken on wrist action shaker for 8 hours. The solubility profile of *Thuja occidentalis* are shown in table-24

The approximate solubility of substances are indicated by the descriptive term in the accompanying table.

**Solubility standard value**

| Descriptive term | Parts of solvent required for 1 part of solute |
|---|---|
| Very soluble | Less than 1 |
| Freely soluble | From 1 to 10 |
| Soluble | From 10 to 30 |
| Sparingly soluble | From 30 to 100 |
| Slightly soluble | From 100 to 1000 |
| Very slightly soluble | From 1000 to 10,000 |
| Practically insoluble or Insoluble | Greater than or equal to 10,000 |

**Table- 6**

**Caliberation curve for extract**

**Preparation of phosphate buffer pH 6.8**

Phosphate buffer pH 6.8 was prepared according to the following methods (I.P.1996). Disodium hydrogen phosphate (6.8g) was dissolved in 500ml of distilled water and sodium hydroxide (1g) was dissolved in distilled water from that 224ml of solution was taken and added with above solution and volume was made up to 1 liter with distilled water. The pH was adjusted to 6.8 prior to quantitative estimation.

**Preparation of Calibration Curve of *Thuja occidentalis* in Phosphate buffer pH 6.8**

A stock solution was prepared by dissolving 100 mg of crude ethanolic extract of *Thuja occidentalis* in 100 ml phosphate buffer of pH 6.8. It was further diluted to obtain the concentration of 5, 10, 15, 20, 25, 30 microgram of the drug per ml. The absorbances of these solutions were determined at 361 nm using UV spectroscopy and given in Figure-8 and Table-25.

The standard curve was constructed between the absorbance and concentration. This standard curve was linearly regressed and statistical parameter related it was derived.

**FABRICATION OF PHYTOSOME**

The phytosomal formulation of *Thuja occidentalis* were prepared by Thin Film Hydration Technique using Rotary Film Evaporation method.

Appropriate amount of cholesterol and phosphoditylcholine were taken in round bottom flask and both were dissolved by addition of 5ml of chloroform and 5ml of methanol. The flask was rotated at above 1.5cm above a water bath at 60 C ± 2C under pressure, until all the organic phase evaporated and a thin layer was formed on the wall of the round bottom flask.

The crude extract of *Thuja occidentalis* was dissolved in 20 ml of distilled water and it was hydrated with dried layers of cholesterol and phosphotidylcholine in the round bottom flask. and this mixture again rotated in a water bath at 60 C ± 2 C for 1 hour.

The phytosome vesicle containing *Thuja occidentalis* were subsequently formed, further subject to ultra sonication at 5° C to 10° C for 15 minutes

**Phytosome preparation flow chart**

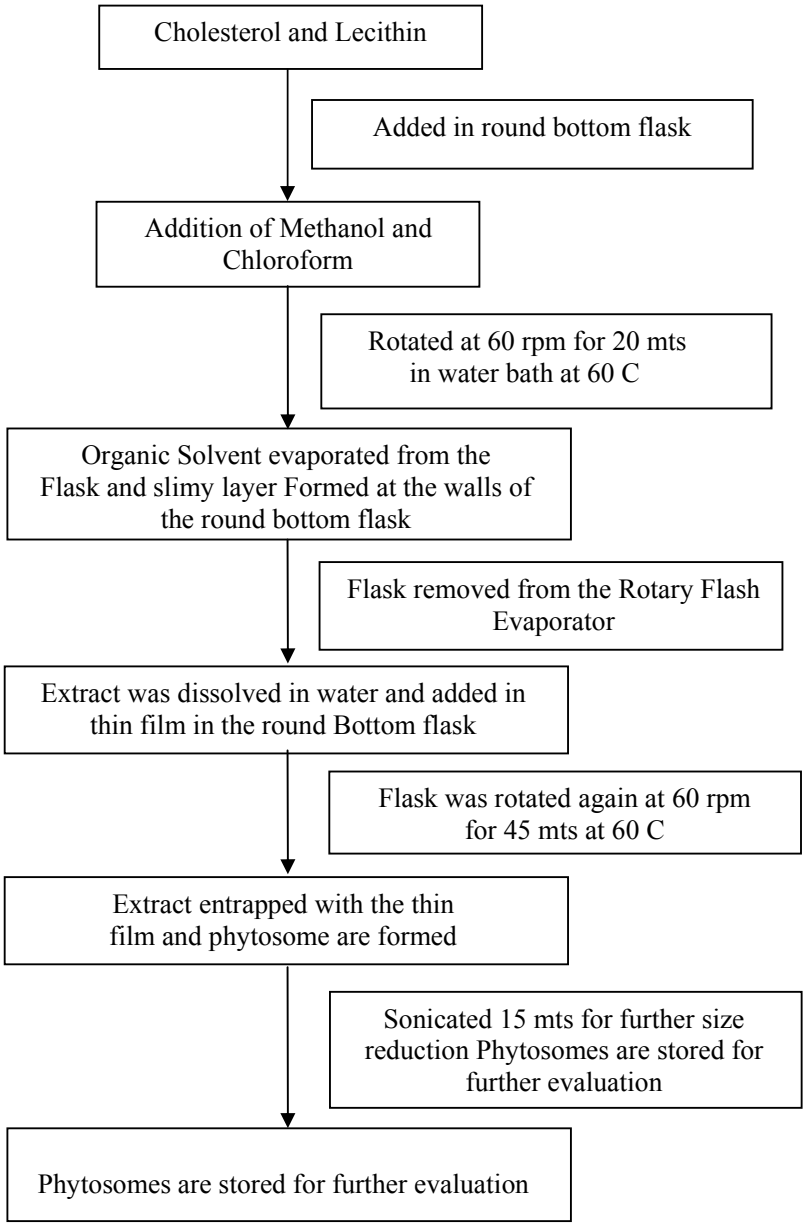


**Figure-6**

The prepared phytosomes were stored in a refrigerator for further evaluation. During this fabrication of phytosomal formulation, various parameters were optimized to get a stable formulation.

**OPTIMIZATION PARAMETERS**

The effect of different temperatures was seen on the formation of phytosomal vesicles

**Optimization of temperature during Phytosomal preparation**

| S.No | Temperature | Observation |
|---|---|---|
| 1 | Below 60° C | Irregular shaped vesicles |
| 2 | 60° C ± 2° C | Spherical vesicles formed |
| 3 | 80° C ±2°C | Vesicles are less and irregular |

**Table-7**

**Optimization of temperature during Ultrasonication**

| S.No | Temperature | Observation |
|---|---|---|
| 1 | 5° C to 10° C | Size of vesicles decreases |
| 2 | 10° C to 20 C | Numerous vesicles of Moderate Size |
| 3 | Room temperature | Large Size Vesicles |

**Table-8**

**Optimization of Rotating speed**

| S.No | Rpm | Observation |
|---|---|---|
| 1 | 50 ± 5 | Clumps |
| 2 | 100 ± 5 | Spherical vesicles |
| 3 | >100 | Irregular shaped vesicles |

**Table-9**

**Optimization of Amount of Solvent**

| S.No | Amount Of Methanol And Chloroform | Observation |
|---|---|---|
| 1 | 1:2 (5 ml : 10ml) | Thick, non uniform film |
| 2 | 1:1 (5 ml : 5 ml) | Thin uniform film |
| 3 | 2:1 (10 ml : 5 ml) | Take mmore time, less vesicle |

**Table- 10**

**Effect of hydration liquid on Drug Entrapment Efficiency**

| S.No | Hydration Solvent | Observation |
|---|---|---|
| 1 | 0.1 M Hcl | Low |
| 2 | Water | High entrapment efficiency |
| 3 | PBS 7.4 | Medium |

**Table- 11**

**Optimization of Ultrasonication time**

| S.No | Time in Minutes | Observation |
|---|---|---|
| 1 | 10 | Incomplete and large sized vesicles |
| 2 | 20 | Spherical vesicles |
| 3 | 30 | Broken vesicles |

**Table-12**

**Optimization ratio of Drug Vs Cholesterol and Lecithin**

| S.No | Formulation Code | Drug | Cholesterol | Lecithin |
|---|---|---|---|---|
| 1 | F1 | 1 (10 mg) | 1.5 (15 mg) | 4 (40 mg) |
| 2 | F2 | 1 (10 mg) | 1.5 (15 mg) | 5 (50 mg) |
| 3 | F3 | 1 (10 mg) | 1.5 (15 mg) | 6 (60 mg) |

**Table-13**

**Optimized formula – Trial formulation**

| S.No | Formulation Code | Drug (mg) | Cholesterol (mg) | Lecithin (mg) | Methanol (ml) | Chloroform (ml) | Water | Drug: Cholesterol: Lecithin |
|---|---|---|---|---|---|---|---|---|
| 1 | F1 | 10 | 15 | 40 | 5 | 5 | 20 | 1:1.5:4 |
| 2 | F2 | 10 | 15 | 50 | 5 | 5 | 20 | 1:1.5:5 |
| 3 | F3 | 10 | 15 | 60 | 5 | 5 | 20 | 1:1.5:6 |

**Table-14**

**COMPATIBILITY STUDIES**

Fourier infrared spectroscopy (FT-IR) was performed with ethanolic extract of *Thuja occidentalis* to find to find any interaction between extract and the Excipients.

**INFRARED SPECTRAL ASSIGNMENT**

Infrared spectrum of any compound or drug gives information about the groups present in that particular compound. IR spectrum of *Thuja occidentalis* extract was taken out on Perkin Elmer IR Spectrophotometer. Various peaks in IR spectrum were interpreted for the presence of different groups.

# CHAPTER-4

# CHARACTERIZATION OF PHYTOSOME

## Morphological Studies

The Phytosomes were subjected to SEM examination for characterizing shape and surface morphology

## Scanning Electron Microscopy

Phytosomes were characterized by SEM (Hitachi model S-3000 H, Japan). The resolution of 3.5nm was used with secondary electron image display. The Phytosomes were coated with gold palladium alloy (150-250A) using a sputter coater. The coater was operated at 2.2kv, 20MV, 0.1 torr (argon) for 90 sec at an accelerating voltage of 15 kv. The results are given in Figure-16

## Particle Size

The phytosomes were subjected to microscopic examination (SEM) for characterizing size. The results are shown in Figure-17

## Size Distribution of Phytosomes

Phytosomes were subjected to Laser Particle Counter (L.P.C) for characterizing size distribution of phytosomes. The results are shown in Table-33

It shows the particle range of 20-200 nm for optimized formulation. The average mean particle size was 100nm.

## Drug Content Analysis

Formulated phytosome were centrifuged for 25° C at 2500rpm for 30 mts to separate pure unentraped phytoconstituent from the formulation

When free drug was seperated, vesicles were lysed using equal volume of Triton-X 100(0.1% vol/vol) and analysed for drug content. The absorbance of the

solution was measured by UV spectrophotometer (Shimadzu 1700) at 361nm against blank to determine its encapsulation efficacy. The results are shown in Table- 35 and Figure- 20

The drug content of phytosomes was calculated according to the following equation.

$$\text{Drug content} = \frac{\text{Actual drug content in Phytosome}}{\text{Theoretical drug content}} \times 100$$

**Drug Entrapment Efficiency**

The entrapment was determined by dialysis method. Dialysis membrane 70 (Himedia, Mumbai, India) was immersed in glyserine overnight before dialysis to ensure complete wetting of the membrane. 10 ml of drug loaded vesicles was placed in membrane which was tied to one end of the open ended cylinder, which was then placed in 500ml distilled water. This receiver medium stirred with a magnetic stirrer. The medium was changed with fresh medium at half an hour interval for atleast 3 to 5 times to remove unentrapped drug and impurities

When free drug was separated, vesicles were lysed using equal volume of Triton-100 (0.1% vol/vol) and analysed for drug content.

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{Amount of drug released from lysed phytosome}}{\text{Amount of drug found in sample}} \times 100$$

**In Vitro Drug Release Studies**

**Cellophane Membrane Treatment**

Cellophane membrane was boiled in the distilled water for 1 hr and washed with fresh distilled water for three times and kept in ethanol for 24 hrs. It was washed with distilled water and treated with 0.3% sodium sulphite and soaked in distilled water for 2min at 60°C followed by acidified with 0.2% sulphuric acid. Finally the membrane was dipped in boric buffer (pH 9) till it is used for permeation study[63].

**Drug Permeation Studies**

The *in vitro* release rate of Phytosomes were evaluated by open ended tube through the cellophane membrane method using PB pH6.8 as diffusion medium up to 12 hours studies. The cellophane membrane is tided in one end of the tube and then immersed in the receptor compartment containing 400ml of PB pH6.8. Which was stirred at medium speed and maintained at 37°C±2°C. Samples were withdrawn at regular time intervals and the same volume was replaced by fresh diffusion medium. The samples were analyzed using UV – visible spectrophotometer (Shimadzu UV1700) set at 361 nm.

**Release Kinetics**

Data obtained from invitro release studies were fitted to various kinetic equations. The kinetic models used are zero order equations (Q=k0t), First order equation {ln (100 – Q)=ln Q – klt}, Higuchi equation($Q=kt^{1/2}$), Hixson and crowell model $Qt^{1/3}$ Vs t and $Qt^{2/3}$ Vs – Modified root cube equation. Further, to find out the mechanism of drug release, first 60% drug release was fitted in Korsmeyer and peppas equation ($Q=kpt^{n}$). where, Q is the percent of the drug release at time t and k0 and kt are the coefficients of the equations and n is the release exponent. The n value is used to characterize different release mechanism.

The order of drug release can be assessed by graphical treatment of drug release data.

A plot of Cumulative % drug release versus time would be linear if the drug release follows zero order (ie. Concentration independent release).

A plot of log of % remaining drug versus time would be linear, if the drug release follows first order (ie. Concentration dependent release)

The linear equation for zero order drug release plot is:

$$Ct = C0 - Kt$$

Where,

Ct = concentration remaining at time t,

Co = original concentration,

t = time,

K = release rate

The linear equation for first order release plot is

$$\text{Log } C = \frac{\log C_0 - Kt}{2.303}$$

A matrix device as the name implies, consists of drug dispersed homogeneously through out a polymer matrix.

In this model, drug in the out side layer exposed to the bathing solution is dissolved first and than diffuses out of the matrix. This process continues with the interface between the bathing solution and the solid drug moving towards the interior. Obviously, for this system to be diffusion controlled, the rate of dissolution of drug particles with in the matrix must be much faster than the diffusion rate of dissolved drug leaving the matrix.

Hydrophilic matrix tablets contain a water swellable polymer. On contact with gastric juices the tablet surface gels, impeding further liquid penetration into

the tablet core and providing a rate controlling layer. Dissolution occurs at the gel core interface and drug diffuse out through the gelled layer.

Drug release is controlled by penetration of water through a gel layer produced by hydration of the polymer and diffusion of drug through the swollen, hydrated matrix, in addition to erosion. The extent to which diffusion or erosion controls release depends on the polymerration.

Mechanism of release from erodible matrix has been described by Hopfenberg. A simple expression describing release from erodible is

$$[1 - Mt / M]^{1/3} = 1\text{-}kt$$

Where,

Mt = mass of drug release at time t,

M = mass release at the infinite time,

K = rate of erosion,

t = time

Thus a plot of $[1 - Mt / M]^{1/3}$ versus the time will be linear. If the release of drug from the matrix is erosion controlled.

In order to ascertain whether the drug release occurs by diffusion or erosion, the drug release data was subjected to following modes of data treatments.

1) Amount of drug release versus square root of time (Higuchi Plot).
2) $[1 - Mt / M]^{1/3}$ versus time.

**Incorporation of Selected Formulation into Gel Base**

Selected phytosomal formulation were incorporated into Sodium alginate Gel base to form a gel. And it is evaluated the following parameters against marketed thuja gel(MTG)[6].

- pH
- Viscosity
- Drug content analysis
- Spreadability
- Extrudability

**pH**

pH of the formulations were measured by using a digital type pH meter (Elico Make) by dipping the electrode completely in the Phytosomal Thuja gel(PTG) and Marketed Thuja Gel(MTG) so as to cover the electrode and read out the pH[8].

**Viscosity**

The viscosity of the prepared PTG and MTG was measured using Brookfield viscometer (R V 1 Brookfield Viscometer) at a controlled temperature.

**Drug content**

A specific quantity (1.0gm) of PTG formulation and MTG formulation was dissolved in distilled water and the solution is filtered through the Whatman filter paper. The absorbance of the solution was measured by UV spectrophotometer (Shimadzu 1700) at 361nm against blank[8].

**Spreadability**

One of the criteria for a gel to meet ideal quality is that it should possess good spreadability. Adout 1gm of Phytosomal Thuja Gel(PTG)and Marketed Thuja Gel (MTG) was weighed and kept at the center of the glass plate (10×10

cm) and another glass plate is placed over it carefully, 2 kg weight was placed at the center of the plate (avoid sliding of the gel). The diameter of the gel in cms, after 30 minutes was measured[8]. The spreadability was calculated by using the formula

$$\text{Spreadability} = \frac{\text{Weight tied to the upper side X length of the glass slides}}{\text{Time taken in seconds}}$$

**Tube extrudability**

It is a useful empirical test to measure the force required to extrude the material from a tube. The formulation under study was filled in a tube with nasal tip of 5 mm opening tube extrudability was then determined by measuring the amount of gel extruded through the tip when a pressure was applied on the tube gel.

**Invitro Drug Release studies**

**Cellophane Membrane Treatment**

Cellophane membrane was boiled in the distilled water for 1 hr and washed with fresh distilled water for three times and kept in ethanol for 24 hrs. It was washed with distilled water and treated with 0.3% sodium sulphite and soaked in distilled water for 2 min at 60◦C followed by acidified with 0.2% sulphuric acid. Finally the membrane was dipped in boric buffer (pH 9) till it is used for permeation study.

**Drug Permeation Studies**

The *in vitro* release rate of PTG and MTG were evaluated by open ended tube through the cellophane membrane method using Phosphate Buffer pH 6.8 as diffusion medium up to 12 hours studies. The cellophane membrane is tided in one end of the tube and then immersed in the receptor compartment containing 400ml of Phosphate Buffer pH 6.8. Which was stirred at medium speed and

maintained at 37°C ± 2°C. Sample were withdrawn at regular time intervals and the same volume was replaced by fresh diffusion medium. The samples were analyzed using UV – visible spectrophotometer (Shimadzu UV 1700) set at 361nm

**Skin irritation Studies**

Skin irritation test was performed on 3 rabbits weighing between 2 – 3.5 kg. Placebo gel was used as control. The *Thuja occidentalis* Phytosome loaded gel was used as a test sample. Test was conducted unbraided skin of rabbits. The control was placed on left dorsal surface of each rabbit, whereas test formulation were placed on identical side of the right dorsal surface of the rabbits, the gel formulation were removed after 24 hours with the help of an alcohol swab and skin was examined for erythma/oedema[9].

**Stability Testing**

Nowadays, Stability testing has become an integral part of formulation development. It generates information on which proposal for shelf life of drug or dosage form and their recommended storage conditions are based.

**Definition**

Stability of a pharmaceutical preparation can be defined as "the capability of a particular formulation (dosage form or drug product) in a specific container/closure system to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications throughout its shelf life[9].

**Purpose of Stability Testing**

- To ensure efficacy, safety and quality of active drug substances and dosage forms,
- To establish shelf life or expiration period and to support label claims.

# CHAPTER-5

# RESULTS AND DISCUSSION

**Phytochemical Analysis**

**Phytochemical analysis for Alkaloids**

| S.No | Test for Constituents | Observation | Inference |
|---|---|---|---|
| 1 | Extract + Mayer's reagent | Cream precipitate | Presence of Alkaloids |
| 2 | Extract + Dragondarffs reagent | Orange precipitate | Presence of Alkaloids |

**Table-15**

**Phytochemical analysis for Corbohydrates**

| S.No | Test for Constituents | Observation | Inference |
|---|---|---|---|
| 1 | Molisch Test; Extract Filtrate + 3 drops of alcoholic α- Napthol + 2 ml Sulphuric acid | Violet colour ring formed | Presence of Corbohydrate |
| 2 | Fehling's Test; Extract Filtrate + 1ml Fehling's reagent and heated | Reddish Orange precipitate | Presence of Corbohydrate |

**Table-16**

**Phytochemical analysis of Glycoside**

| S.No | Test for Constituents | Observation | Inference |
|---|---|---|---|
| 1 | Borntrager's Test; Extract filtrate + Chloroform added and then chloroform layer is separated, then added dilute ammonia solution | No colour change | Presence of glycoside |

**Table-17**

**Phytochemical analysis of Phytosterol**

| S.No | Test for Constituents | Observation | Inference |
|---|---|---|---|
| 1 | 1 g of Extract + Dil. Acetic acid + Few drops of Con.Sulphuric acid | Bluish green colour | Absence of phytosterols |

**Table-18**

**Phytochemical analysis of Fixed oil and Fat**

| S.No | Test for Constituents | Observation | Inference |
|---|---|---|---|
| 1 | Extract placed in between two filter paper | Oil stain in the filter paper | Presence of oil and fat |

**Table-19**

**Test for Tannin and Phenolic Compound**

| S.No | Test for Constituents | Observation | Inference |
|---|---|---|---|
| 1 | Extract is treated with 10% lead acetate solution | White precipitate is formed | Presence of phenolic compounds |

**Table-20**

**Phytochemical analysis of Saponin**

| S.No | Test for Constituents | Observation | Inference |
|---|---|---|---|
| 1 | Extract diluted with 20 ml of distilled water and agitated on a cylinder for 15 mts | Formation of foam | Presence of saponin |

**Table-21**

**Phytochemical analysis of Flavonoids**

| S.No | Test for Constituents | Observation | Inference |
|---|---|---|---|
| 1 | Shimoda's Test<br>Extract + Magnesium turnings + Few drops of Con.Hydro chloric acid and boil for 5 minutes | Red colour is obtained | Confirm the Presence of Flavonoids |

**Table-22**

## CHARACTERIZATION OF EXTRACT

### FT-IR of Extract

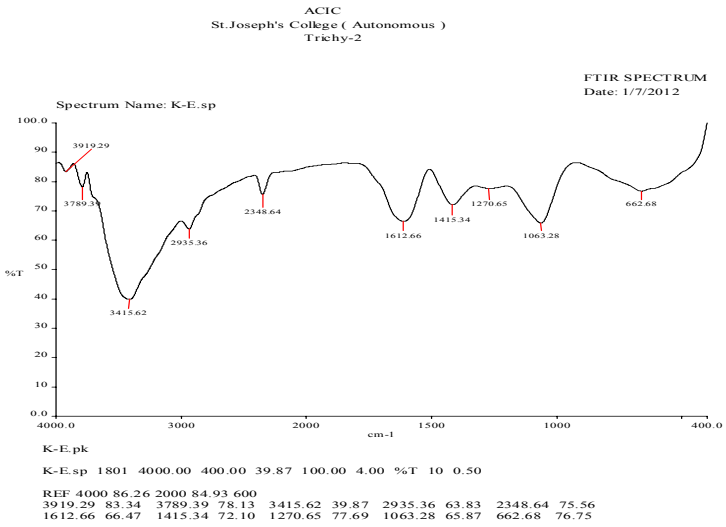


**Figure-6-a**

### Interpretation of Extract

| S.No | Wavelengh | Functional Group |
|---|---|---|
| 1 | 3415 | N-H Stretching |
| 2 | 2935 | C-H Stretching |
| 3 | 2348 | Broad OH |
| 4 | 1612 | N-H Bending (primary amine) |
| 5 | 1415 | $CH_3$-O-R |
| 6 | 1270 | C-O Stretching |
| 7 | 1063 | C-N Stretching |
| 8 | 662 | C-H Out of plane bond |

**Table-23**

**Absorption Maxima**

**Absorption maxima of Extract**

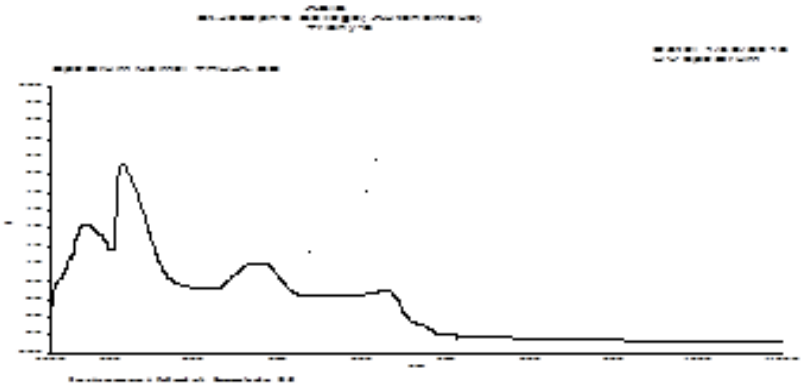

**Figure-7**

The drug solution (1mg/ml) in phosphate buffer pH 6.8 was taken in the cuvette scanned in the range of 230 to 400 nm in a UV spectrophotometer. It exhibits maxima at **361nm**. Therefore, all the further measurements were taken at 361nm

**Solubility profile of Extract**

| S.No | Solvent | Solubility |
|---|---|---|
| 1 | Distilled water | Very soluble |
| 2 | PBS (pH 6.8) | Very soluble |
| 3 | Methanol | Sparingly soluble |
| 4 | Ethanol | Soluble |
| 5 | Chloroform | practically insoluble |
| 6 | Acetone | Practically insoluble |

**Table-24**

**Standard Curve in PBS 6.8**

| S.No | Concentration (µg/ml) | Absorbance at 361nm |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 10 | 0.1843 |
| 3 | 20 | 0.3662 |
| 4 | 30 | 0.4882 |
| 5 | 40 | 0.6442 |
| 6 | 50 | 0.8344 |
| 7 | 60 | 0.9611 |

**Table-25**

**Standard curve of Extract**

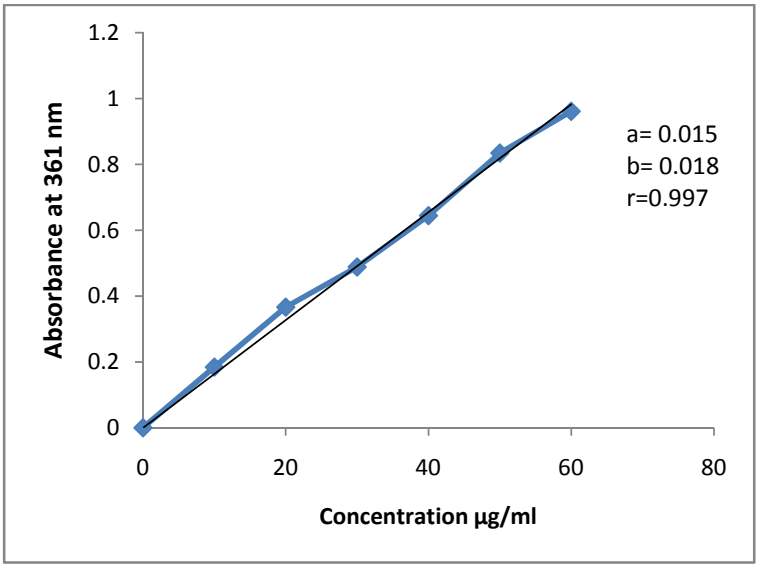


**Figure- 8**

**Compatibility studies**

**FT-IR Spectrum of *Thuja occidentalis***

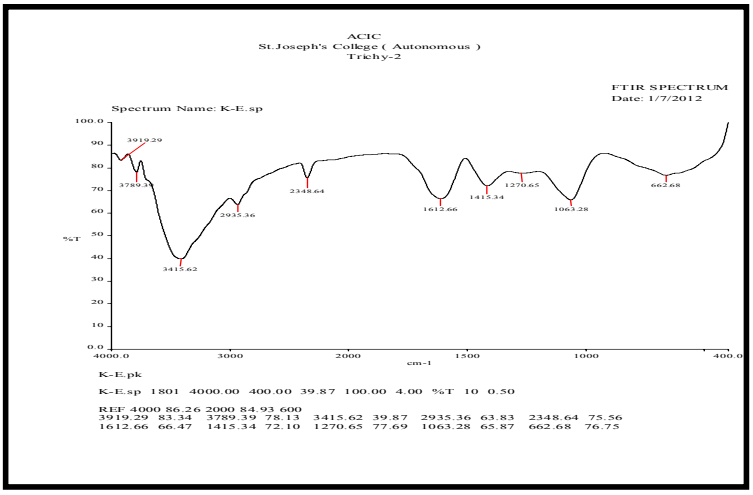


**Figure-9**

**FT-IR Interpretation of *Thuja occidentalis***

| S.No | Wavelengh | Functional Group |
|---|---|---|
| 1 | 3415 | N-H Stretching |
| 2 | 2935 | C-H Stretching |
| 3 | 2348 | Broad OH |
| 4 | 1612 | N-H Bending (primary amine) |
| 5 | 1415 | $CH_3$-O-R |
| 6 | 1270 | C-O Stretching |
| 7 | 1063 | C-N Stretching |
| 8 | 662 | C-H Out of plane bend |

**Table-26**

**FT-IR Spectrum of Cholesterol**

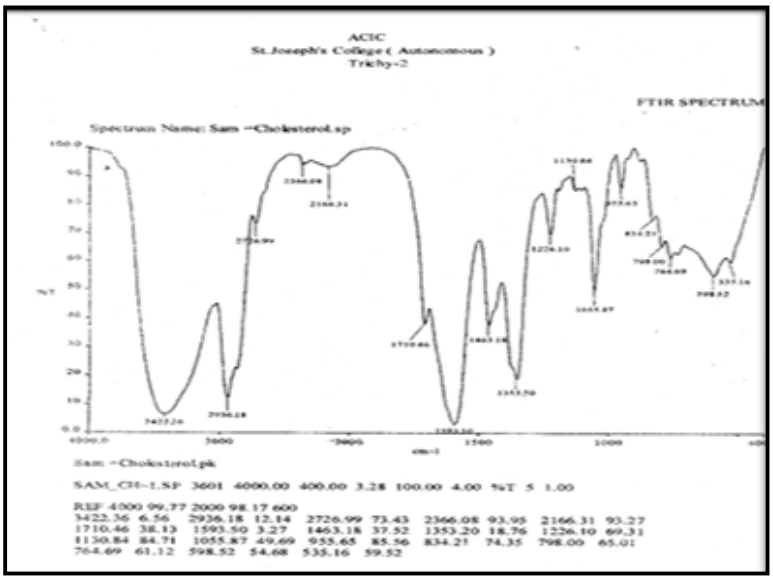


**Figure-10**

**FT-IR Interpretation of Cholesterol**

| S.No | Wave lengh | Functional Group |
|---|---|---|
| 1 | 3422.36 | O-H Stretching |
| 2 | 2936.18 | C-H Stretching |
| 3 | 1710.46 | C=O Stretching |
| 4 | 1353.20-1463.18 | C=N Stretching |
| 5 | 1593.50 | C=O Stretching |
| 6 | 1226.10 | C-O Stretching |
| 7 | 1055.83 | C-C Stretching |

**Table-27**

**FT-IR Spectrum of Phosphotidyl Choline**

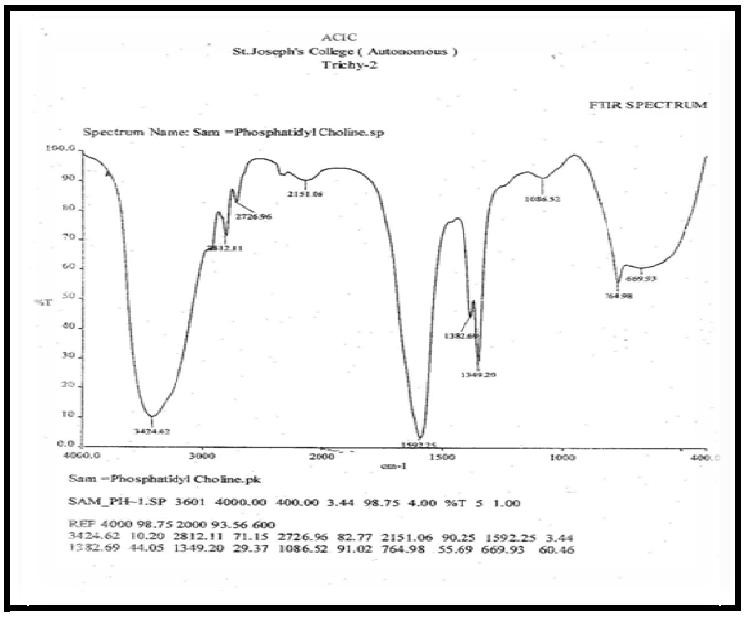


**Figure-11**

**FT-IR Interpretation of Phosphotidyl Choline**

| S.No | Wavelengh | Functional Group |
|---|---|---|
| 1 | 3424.62 | O-H Stretching |
| 2 | 1592.25 | C=O Stretching |
| 3 | 668.34 | C-H Out of plane bend |
| 4 | 1485 | C-H Bending |
| 5 | 1349.20 | C-N Stretching |

**Table-28**

## FT-IR Spectrum of Chloroform

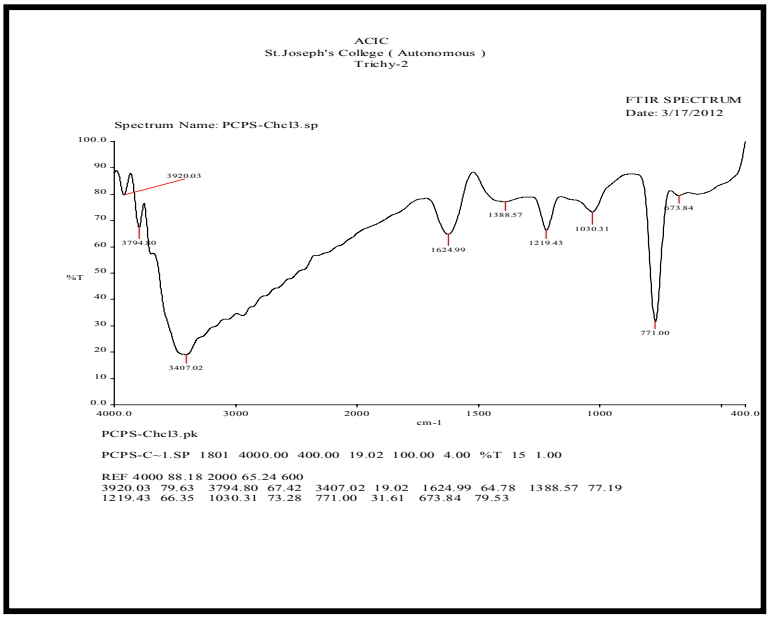


**Figure-12**

## FT-IR Interpretation of Chloroform

| S.No | Wavelengh | Functional Group |
|---|---|---|
| 1 | 3424.62 | O-H Stretching |
| 2 | 1592.25 | C=O Stretching |
| 3 | 668.34 | C-H Out of plane bend |
| 4 | 1485 | C-H Bending |
| 5 | 1349.20 | C-N Stretching |

**Table-29**

## FT-IR Spectrum of Methanol

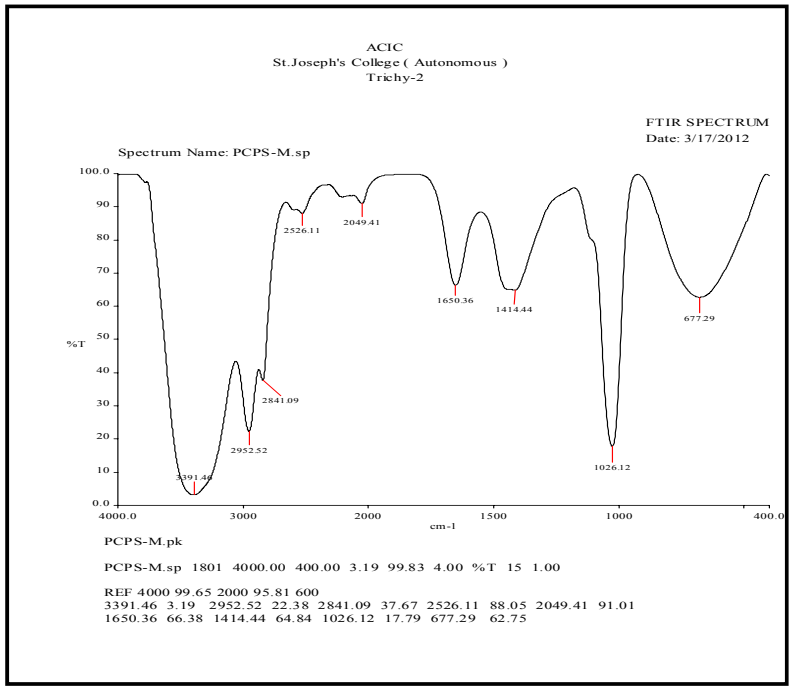


**Figure-13**

## FT-IR Interpretation of Methanol

| S.No | Wavelengh | Functional Group |
|---|---|---|
| 1 | 3415 | N-H Stretching |
| 2 | 2935 | C-H Stretching |
| 3 | 2348S | Broad OH |
| 4 | 1612 | N-H Bending (primary amine) |
| 5 | 1415 | $CH_3$-O-R |
| 6 | 1270 | C-O Stretching |
| 7 | 1063 | C-N Stretching |
| 8 | 662 | C-H Out of plane bend |

**Table-30**

**FT-IR Spectrum of Formulation (Placebo)**

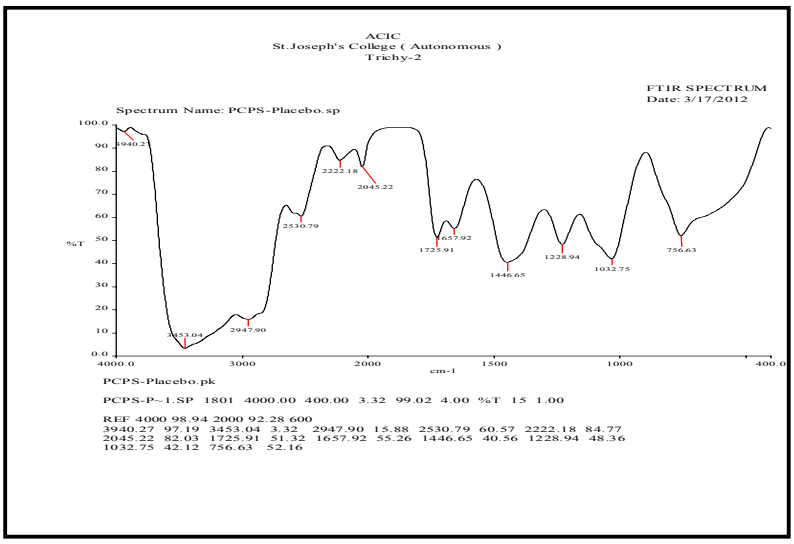


**Figure-14**

**FT-IR Interpretation of Formulation (Placebo)**

| S.No | Wavelengh | Functional Group |
|---|---|---|
| 1 | 3563 | N-H Stretching |
| 2 | 2876 | C-H Stretching |
| 3 | 2186 | Broad OH |
| 4 | 1684 | N-H Bending (primary amine) |
| 5 | 1380 | $CH_3$-O-R |
| 6 | 1265 | C-O Stretching |
| 7 | 1040 | C-N Stretching |
| 8 | 842 | C-H Out of plane bend |

**Table-31**

**FT-IR Spectrum of Formulation**

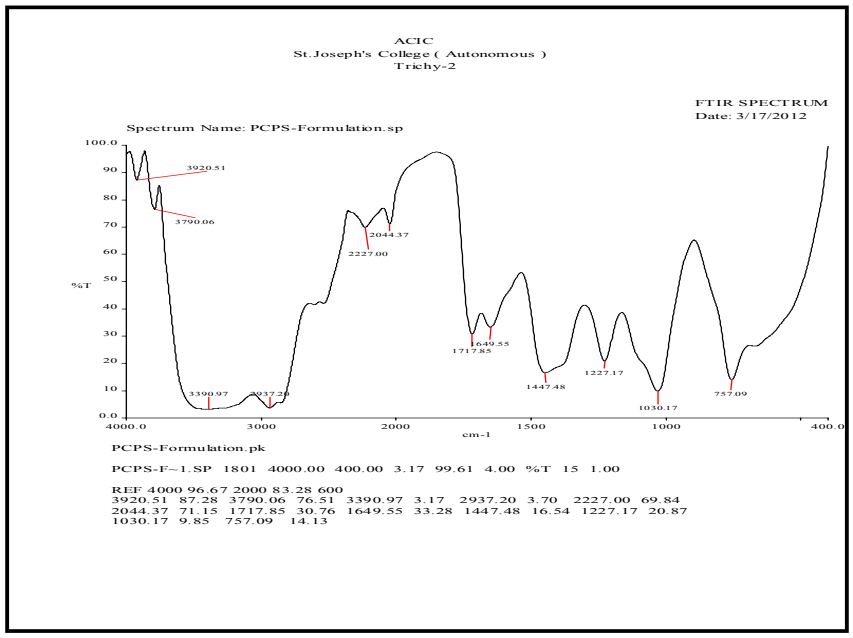


**Figure-15**

**FT-IR- Interpretation of Formulation**

| S.No | Wavelengh | Functional Group |
|---|---|---|
| 1 | 3540 | N-H Stretching |
| 2 | 2852 | C-H Stretching |
| 3 | 2490 | Broad OH |
| 4 | 1464 | N-H Bending (primary amine) |
| 5 | 1280 | $CH_3$-O-R |
| 6 | 1184 | C-O Stretching |
| 7 | 980 | C-N Stretching |
| 8 | 648 | C-H Out of plane bend |

**Table-32**

**SEM Photography of Phytosome DDS**

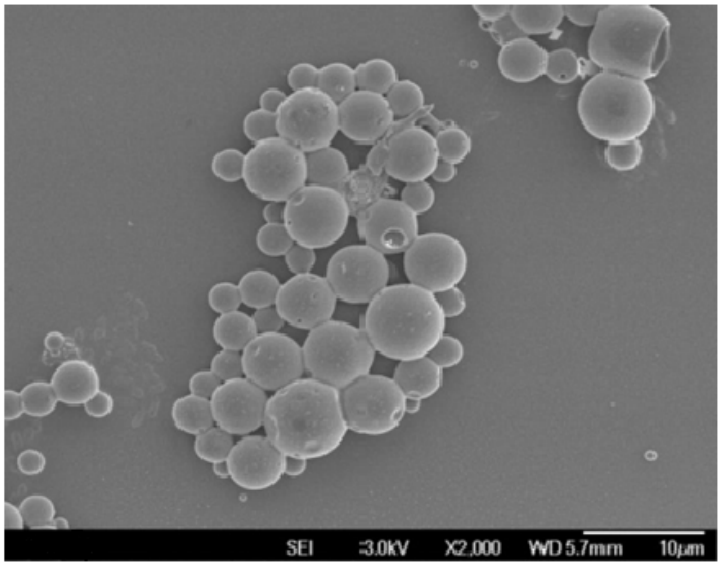


**Figure-16**

**Shape of Thuja occidentalis Phytosome DDS**

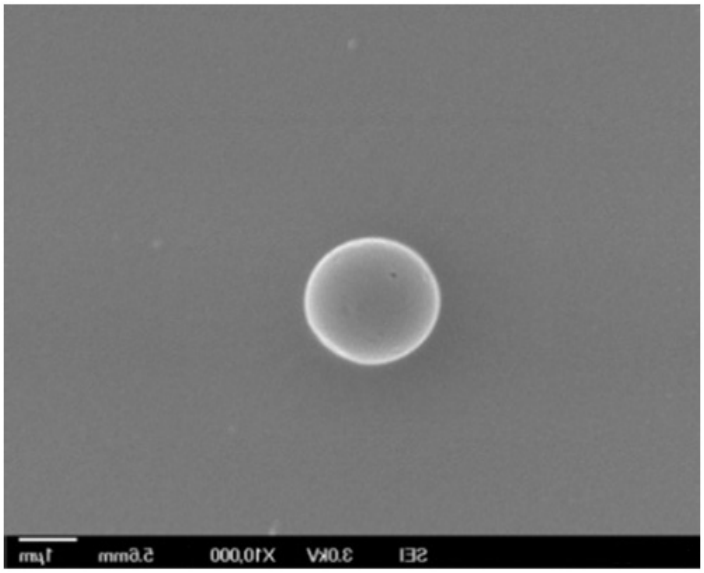

**Figure-17**

**Particle size distribution of Phytosomes**

| S.No | Formulation Code | Average Particle Size Of phytosome(nm) |
|---|---|---|
| 1 | F1 | 260 |
| 2 | F2 | 100 |
| 3 | F3 | 60 |

**Table-33**

**Comparitive Chart of Average Particle Size**

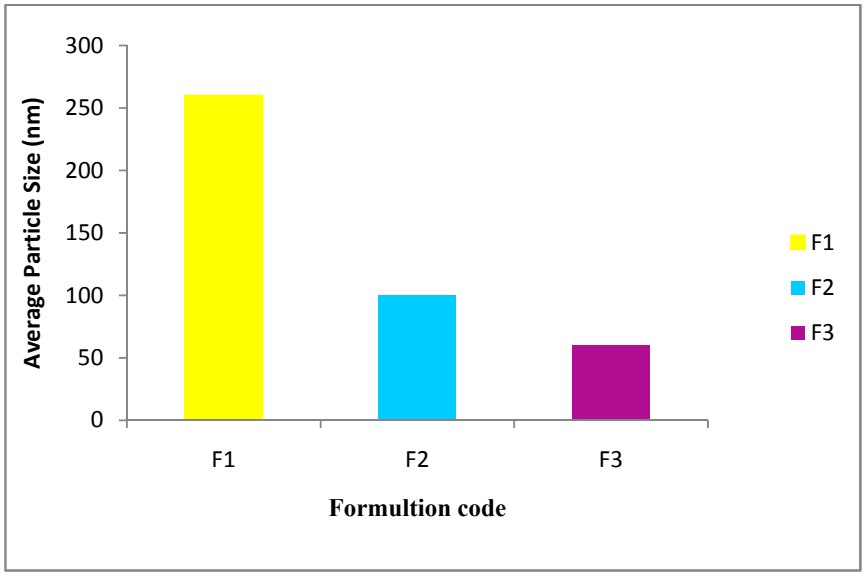


**Figure-18**

**Drug content**

| S.No | Formulation Code | % Drug content |
|---|---|---|
| 1 | F1 | 64 ± 0.5 |
| 2 | F2 | 78 ± 0.5 |
| 3 | F3 | 67 ± 0.5 |

**Table-34**

**Comparative Drug Content**

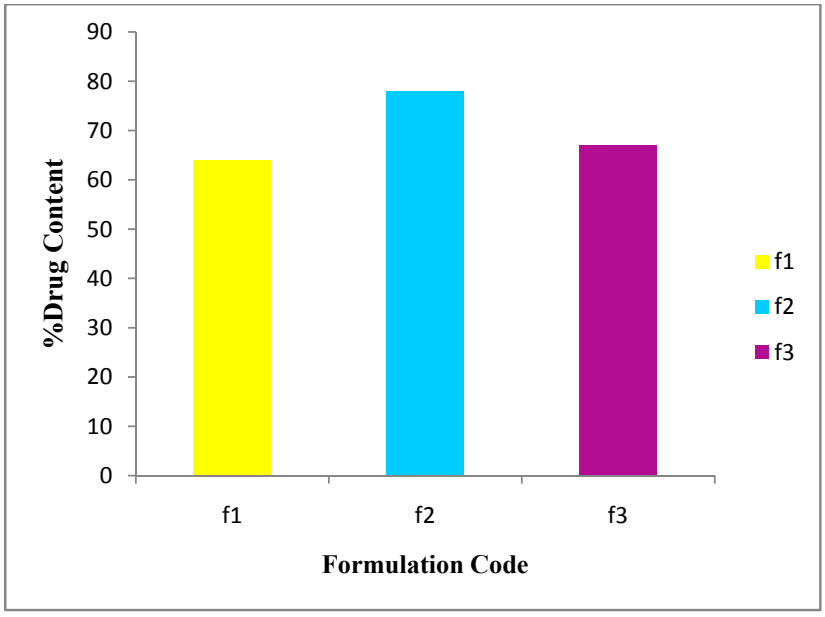


**Figure-19**

**Drug entrapment efficiency**

| S.No | Formulation Code | % Entrapment efficiency |
|---|---|---|
| 1 | F1 | 64 ± 0.5 |
| 2 | F2 | 82 ± 0.5 |
| 3 | F3 | 76 ± 0.5 |

**Table-35**

**Comparison of Drug entrapment efficiency**

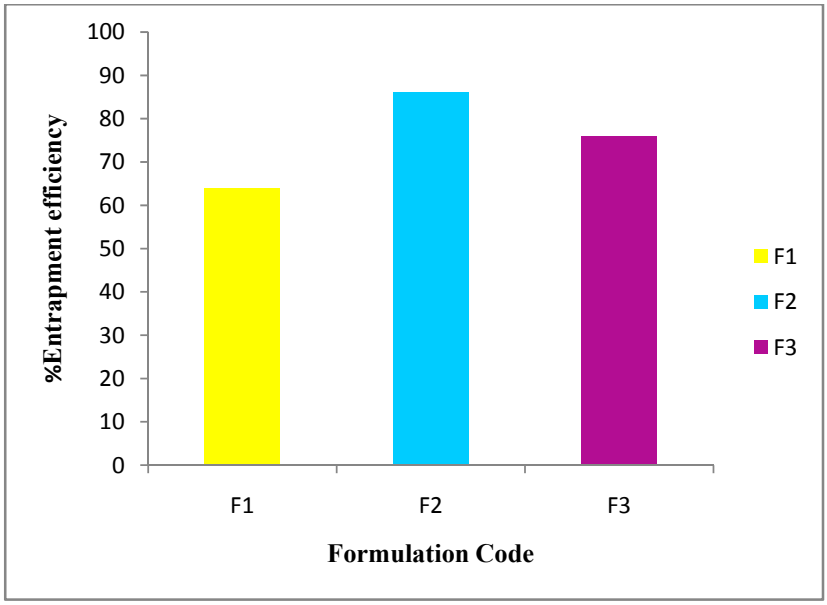


**Figure-20**

## IN VITRO DRUG RELEASE STUDIES

### Invitro drug Release F1

| S.No | Time | Absorbance | Concentration | Amount present | % Amount release |
|---|---|---|---|---|---|
| 1 | 15 | 0.0821 | 3.02580 | 1.2103 | 5.1032 |
| 2 | 30 | 0.1072 | 4.64516 | 1.8580 | 8.5806 |
| 3 | 45 | 0.1172 | 5.29032 | 2.1161 | 12.1612 |
| 4 | 60 | 0.1387 | 6.67741 | 2.6709 | 15.7096 |
| 5 | 90 | 0.1683 | 8.58709 | 3.4348 | 18.3486 |
| 6 | 120 | 0.1836 | 9.57419 | 3.8296 | 21.2967 |
| 7 | 150 | 0.2018 | 10.7483 | 4.2993 | 23.9935 |
| 8 | 180 | 0.2159 | 11.6580 | 4.6632 | 27.6322 |
| 9 | 240 | 0.2216 | 12.0258 | 4.8103 | 28.1032 |
| 10 | 300 | 0.2318 | 12.6838 | 5.0735 | 30.7354 |
| 11 | 360 | 0.2337 | 12.8064 | 5.1225 | 32.2258 |
| 12 | 420 | 0.2379 | 13.0774 | 5.2309 | 38.3096 |
| 13 | 480 | 0.2391 | 13.1548 | 5.2619 | 41.6193 |
| 14 | 540 | 0.2476 | 13.7032 | 5.4812 | 46.8129 |
| 15 | 600 | 0.2498 | 13.8451 | 5.5380 | 55.3806 |
| 16 | 660 | 0.2661 | 14.8967 | 5.9587 | 54.5871 |
| 17 | 720 | 0.2373 | 14.9741 | 5.9896 | 51.8967 |

**Table-36**

**Invitro drug Release F1**

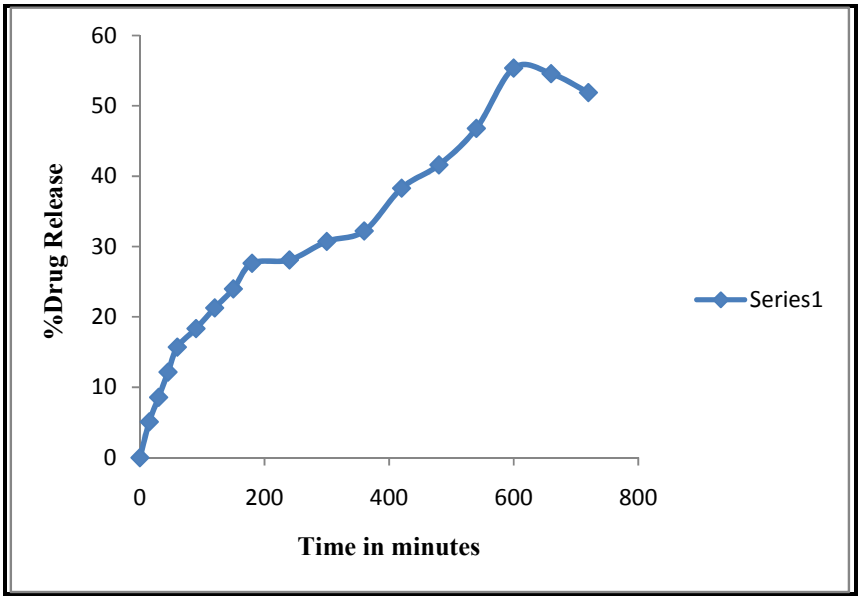


**Figure-21**

**Invitro drug release F2**

| S.No | Time | Absorbance | Conc | Amt Release | %Amt Release |
|---|---|---|---|---|---|
| 1 | 15 | 0.0736 | 2.477419 | 0.9909 | 9.9096 |
| 2 | 30 | 0.0926 | 3.7032 | 1.4818 | 14.8167 |
| 3 | 45 | 0.1011 | 4.2516 | 1.7006 | 17.0064 |
| 4 | 60 | 0.1026 | 4.3483 | 1.7393 | 17.3935 |
| 5 | 90 | 0.1382 | 6.6451 | 2.6580 | 26.5806 |
| 6 | 120 | 0.1511 | 7.4774 | 2.9909 | 29.9096 |
| 7 | 150 | 0.1629 | 8.2386 | 3.2954 | 32.9548 |
| 8 | 180 | 0.1631 | 8.2516 | 3.3006 | 33.0064 |
| 9 | 240 | 0.1821 | 9.4774 | 3.7909 | 37.9096 |
| 10 | 300 | 0.2349 | 12.8834 | 5.1535 | 51.5354 |
| 11 | 360 | 0.2481 | 13.7354 | 5.4941 | 54.9419 |
| 12 | 420 | 0.2482 | 13.7419 | 5.4964 | 54.9677 |
| 13 | 480 | 0.2693 | 15.1032 | 6.0412 | 63.4129 |
| 14 | 540 | 0.2769 | 15.5935 | 6.2374 | 63.3741 |
| 15 | 600 | 0.2839 | 16.0451 | 6.4180 | 64.1806 |
| 16 | 660 | 0.2841 | 16.0580 | 6.4232 | 64.23226 |
| 17 | 720 | 0.2681 | 15.0258 | 6.0103 | 60.10323 |

**Table-37**

**Invitro drug release F2**

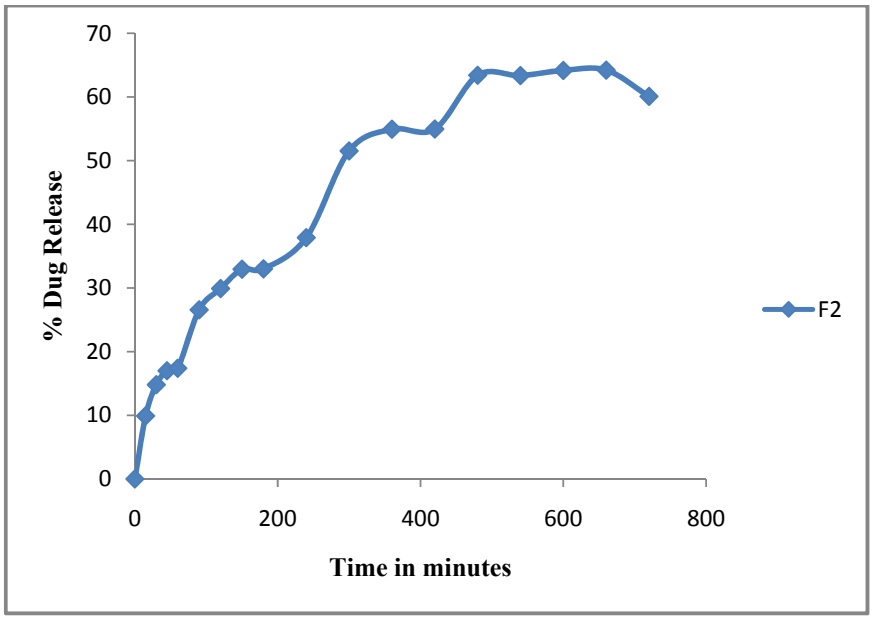


**Figure-22**

**Invitro drug release F3**

| S.No | Time | Absorbance | Concentration | Amount Release | %Amt Release |
|---|---|---|---|---|---|
| 1 | 15 | 0.0683 | 2.135484 | 0.854194 | 8.541935 |
| 2 | 30 | 0.1038 | 4.425806 | 1.770323 | 17.70323 |
| 3 | 45 | 0.1095 | 4.793548 | 1.917419 | 19.17419 |
| 4 | 60 | 0.1385 | 6.664516 | 2.665806 | 21.65806 |
| 5 | 90 | 0.1567 | 7.83871 | 3.135484 | 22.35484 |
| 6 | 120 | 0.1923 | 10.13548 | 4.054194 | 24.54194 |
| 7 | 150 | 0.2009 | 10.69032 | 4.276129 | 26.76129 |
| 8 | 180 | 0.2154 | 11.62581 | 4.650323 | 26.50323 |
| 9 | 240 | 0.2211 | 11.99355 | 4.797419 | 28.97419 |
| 10 | 300 | 0.2318 | 12.68387 | 5.073548 | 30.73548 |
| 11 | 360 | 0.2314 | 12.65806 | 5.063226 | 32.63226 |
| 12 | 420 | 0.2371 | 13.02581 | 5.210323 | 38.10323 |
| 13 | 480 | 0.2411 | 13.28387 | 5.313548 | 40.13548 |
| 14 | 540 | 0.2471 | 13.67097 | 5.468387 | 41.68387 |
| 15 | 600 | 0.2485 | 13.76129 | 5.504516 | 50.04516 |
| 16 | 660 | 0.2716 | 15.25161 | 6.100645 | 53.00645 |
| 17 | 720 | 0.266 | 14.89032 | 5.956129 | 52.56129 |

**Table-38**

**Invitro Drug Release of F 3**

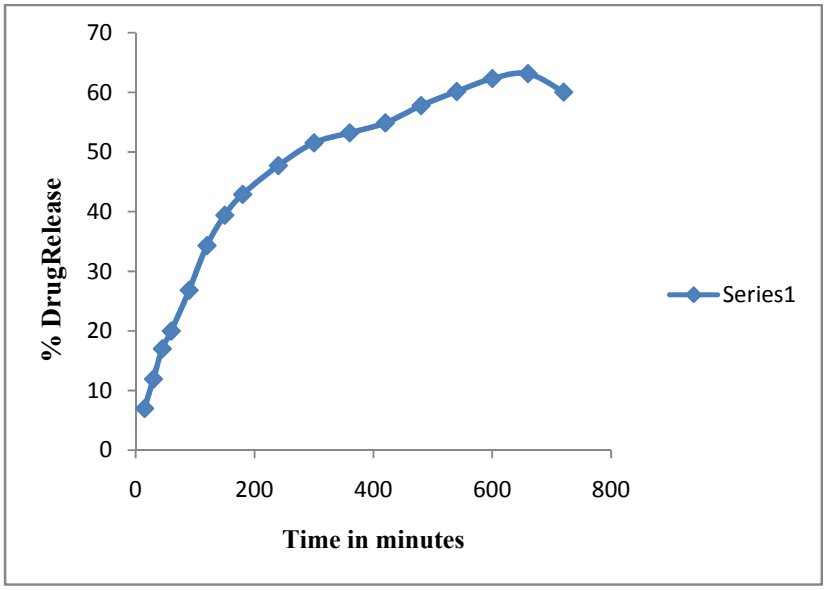


**Figure-23**

**Comparative Invitro Drug Release profile**

| S.No | Time | $F_1$ | $F_2$ | $F_3$ |
|---|---|---|---|---|
| 1 | 15 | 5.1032 | 9.9096 | 8.541935 |
| 2 | 30 | 8.5806 | 14.8167 | 17.70323 |
| 3 | 45 | 12.1612 | 17.0064 | 19.17419 |
| 4 | 60 | 15.7096 | 17.3935 | 21.65806 |
| 5 | 90 | 18.3486 | 26.5806 | 22.35484 |
| 6 | 120 | 21.2967 | 29.9096 | 24.54194 |
| 7 | 150 | 23.9935 | 32.9548 | 26.76129 |
| 8 | 180 | 27.6322 | 33.0064 | 26.50323 |
| 9 | 240 | 28.1032 | 37.9096 | 28.97419 |
| 10 | 300 | 30.7354 | 51.5354 | 30.73548 |
| 11 | 360 | 32.2258 | 54.9419 | 32.63226 |
| 12 | 420 | 38.3096 | 54.9677 | 38.10323 |
| 13 | 480 | 41.6193 | 63.4129 | 40.13548 |
| 14 | 540 | 46.8129 | 63.3741 | 41.68387 |
| 15 | 600 | 55.3806 | 64.1806 | 50.04516 |
| 16 | 660 | 54.5871 | 64.23226 | 53.00645 |
| 17 | 720 | 51.8967 | 60.10323 | 52.56129 |

**Table-39**

**Comparative Invitro Drug Release profile**

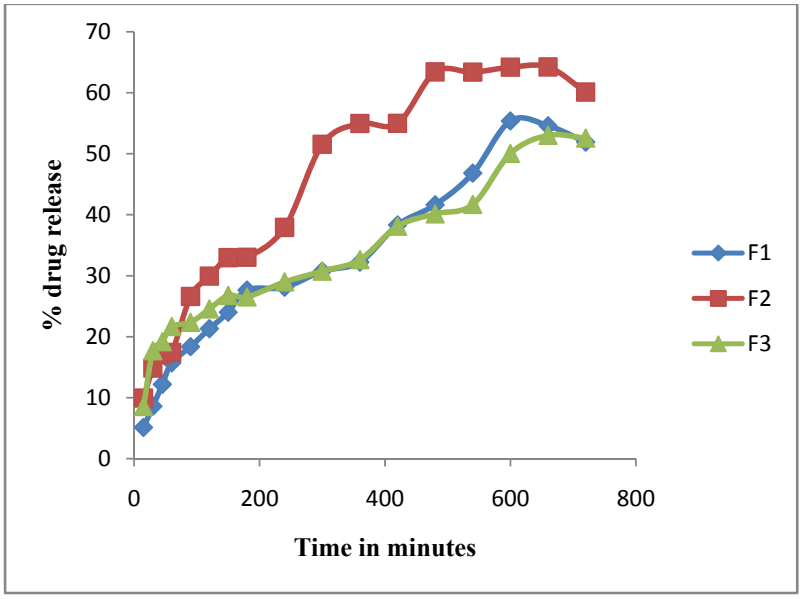


**Figure - 24**

**Release Kinetics**

Table- Parameters of the model equations applied to the release of Thuja occidentalis Phytosomal formulation for Topical administration

**Release Kinetics of Formulation**

| Formulation | Model | $r^2$ | Slope | K |
|---|---|---|---|---|
| F1 | Zero order equation | 0.9708 | 1.6908 | 4.6247 |
| | First order equation | 0.9690 | -0.0184 | 2.0507 |
| | Higuchi model | 0.9735 | 14.536 | -18.61 |
| | Hixson-Crowell | 0.770 | 0.058 | -1.797 |
| | Modified cube root | 0.4462 | 2.054 | -35.30 |
| | Korsmeyer-peppas | 0.964 | 0.864 | 1.474 |
| | | | | |
| F2 | Zero order equation | 0.994 | 0.8764 | 5.1956 |
| | First order equation | 0.9598 | -0.0076 | 2.013 |
| | Higuchi model | 0.9739 | 9.5195 | -12.656 |
| | Hixson-Crowell | 0.803 | 0.031 | -1.818 |
| | Modified cube root | 0.9584 | 5.330 | -18.18 |
| | Korsmeyer-peppas | 0.986 | 0.753 | 1.58 |
| | | | | |
| F3 | Zero order equation | 0.8671 | 4.773 | -1.6277 |
| | First order equation | 0.9137 | -0.0184 | 2.2060 |
| | Higuchi model | 0.939 | 25.83 | -31.00 |
| | Hixson-Crowell | 0.8396 | 0.279 | -0.167 |
| | Modified cube root | 0.8089 | 0.946 | -0.9066 |
| | Korsmeyer-peppas | 0.750 | 0.785 | 1.291 |

**Table-40**

**Release kinetics of F1, F2, F3**

**Release Kinetics of F1**


**Figure - 25**

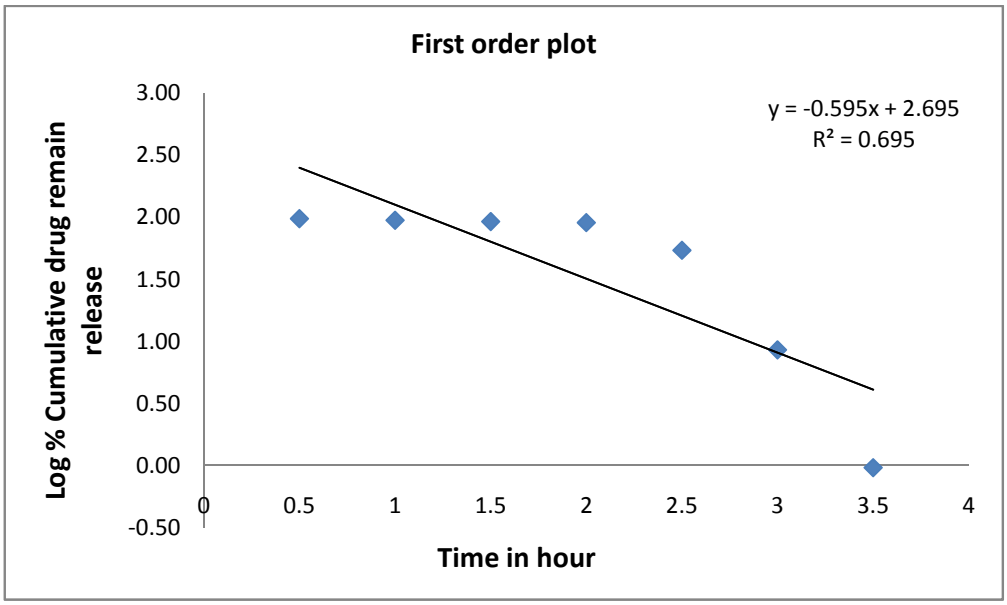


**Figure – 26**

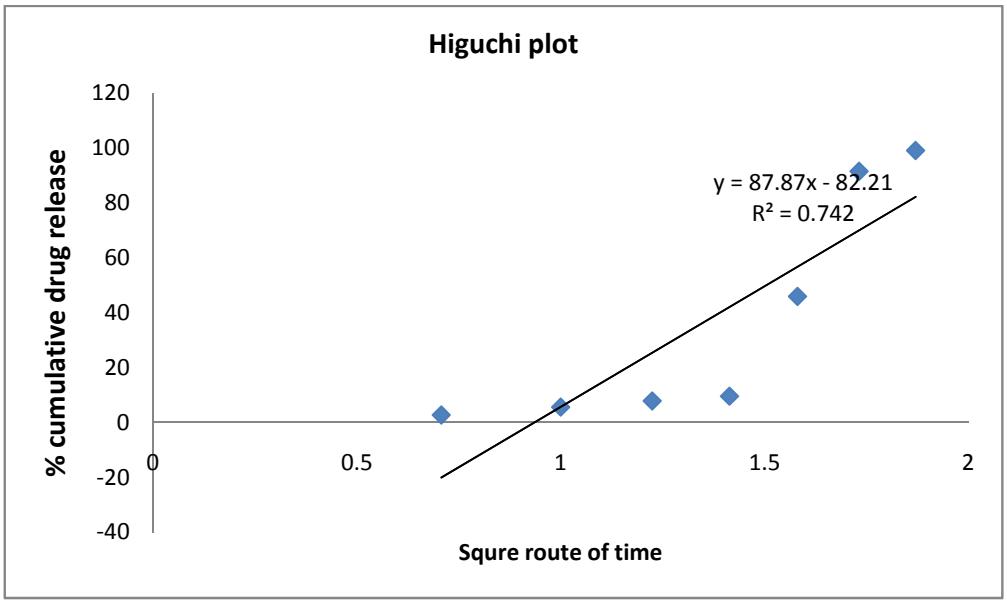


**Figure - 27**

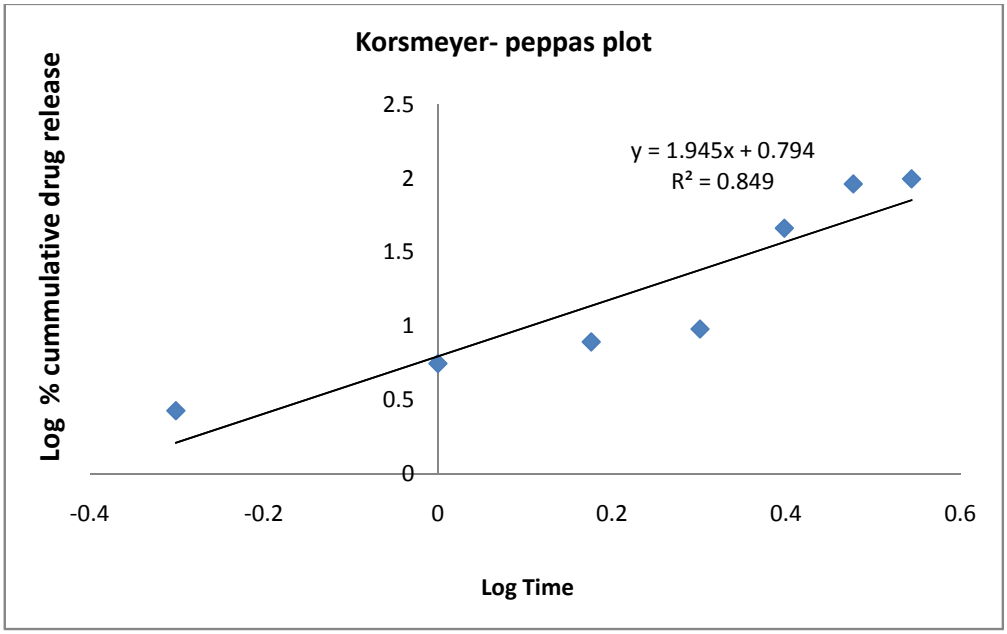


**Figure - 28**

**Release kinetics of F2**

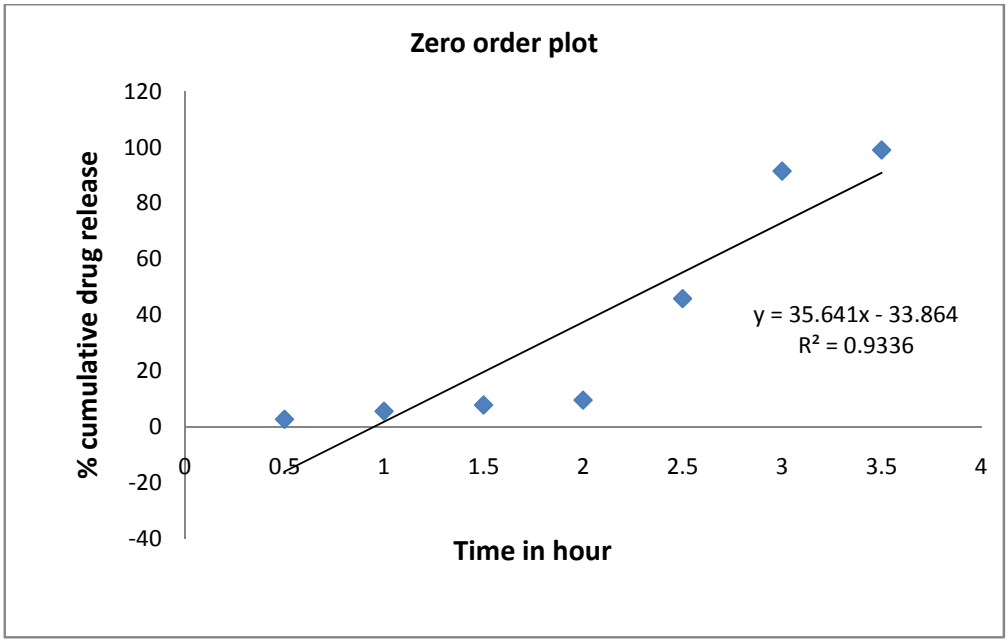


**Figure - 29**

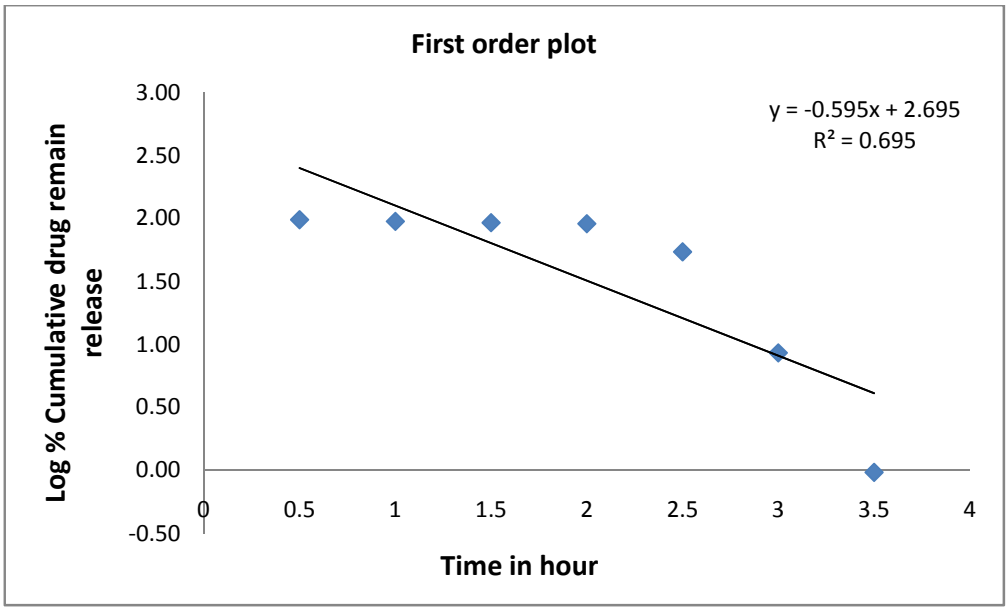


**Figure - 30**

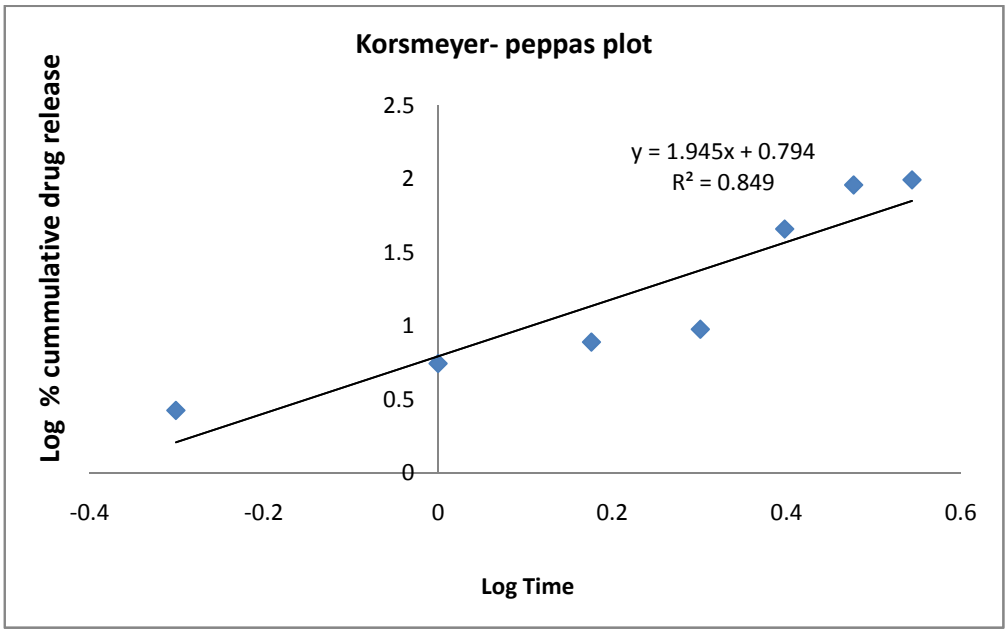


**Figure - 31**

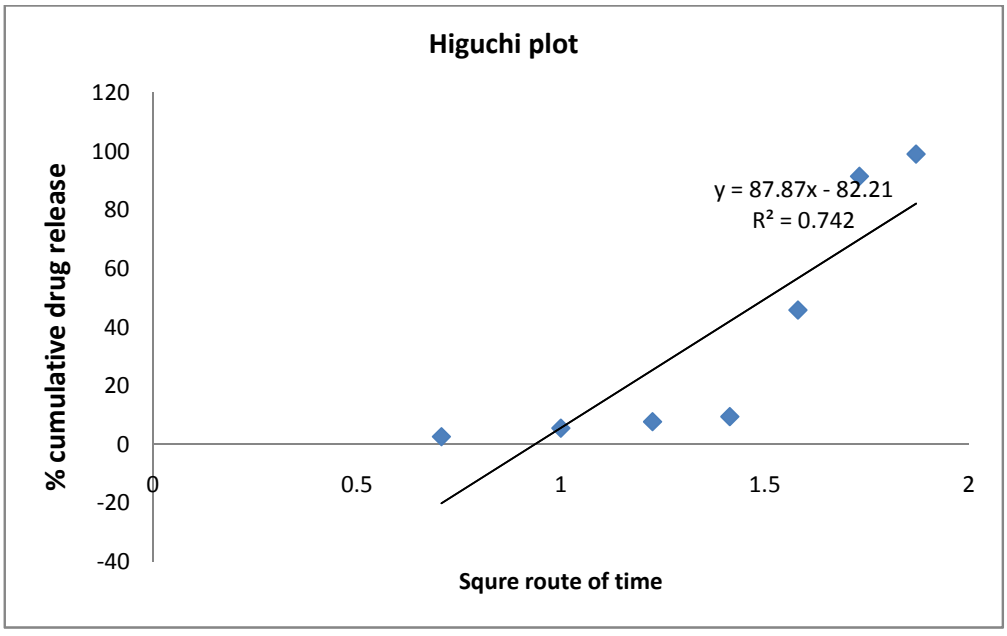


**Figure - 32**

**Release Kinetics of F3**

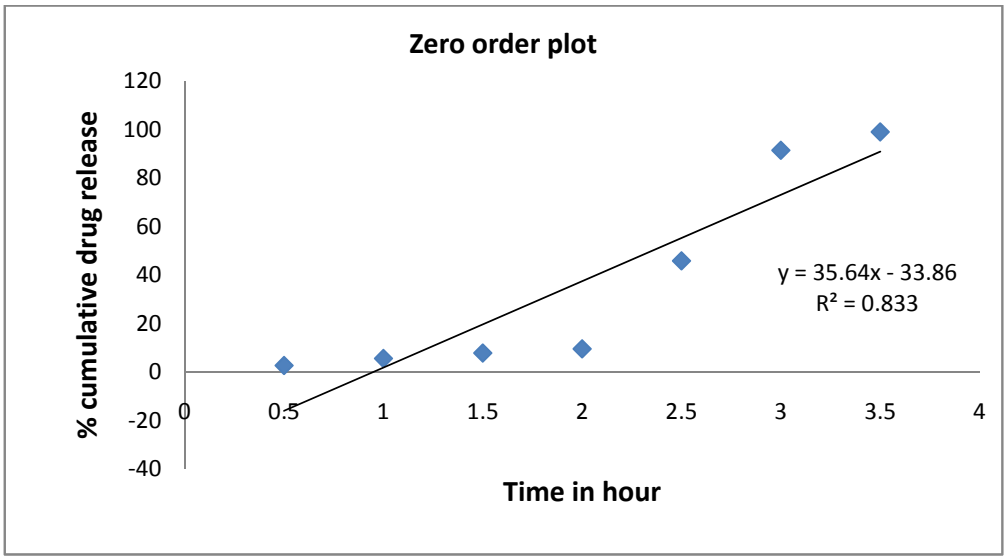


**Figure – 33**

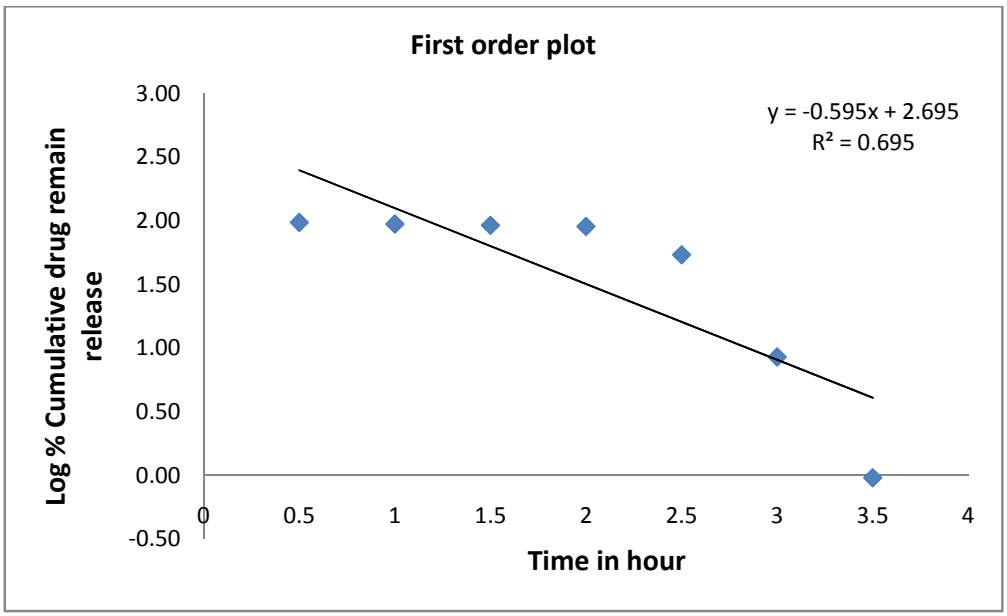


**Figure - 34**

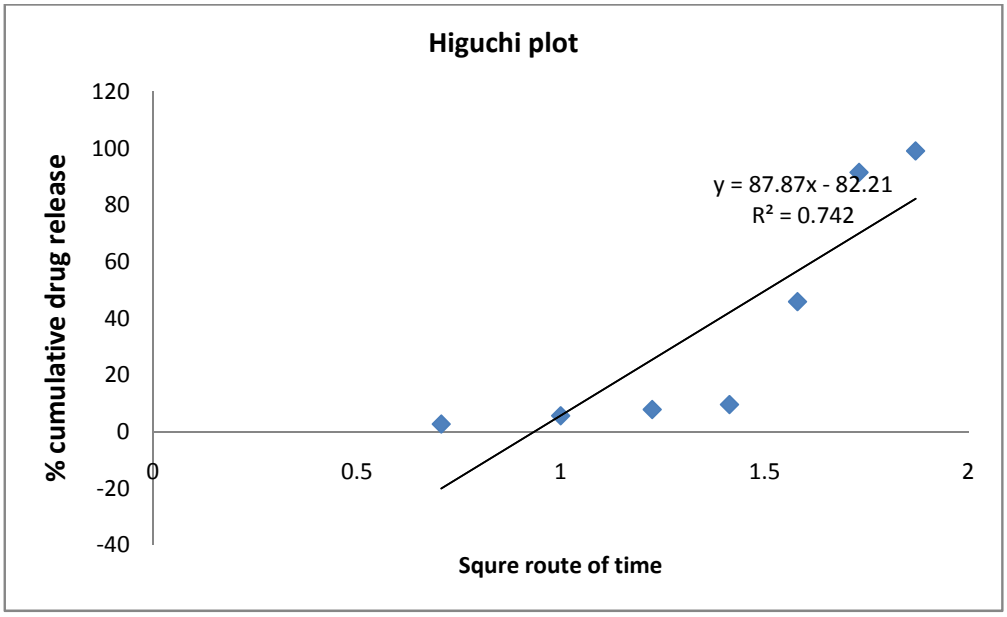


**Figure - 35**

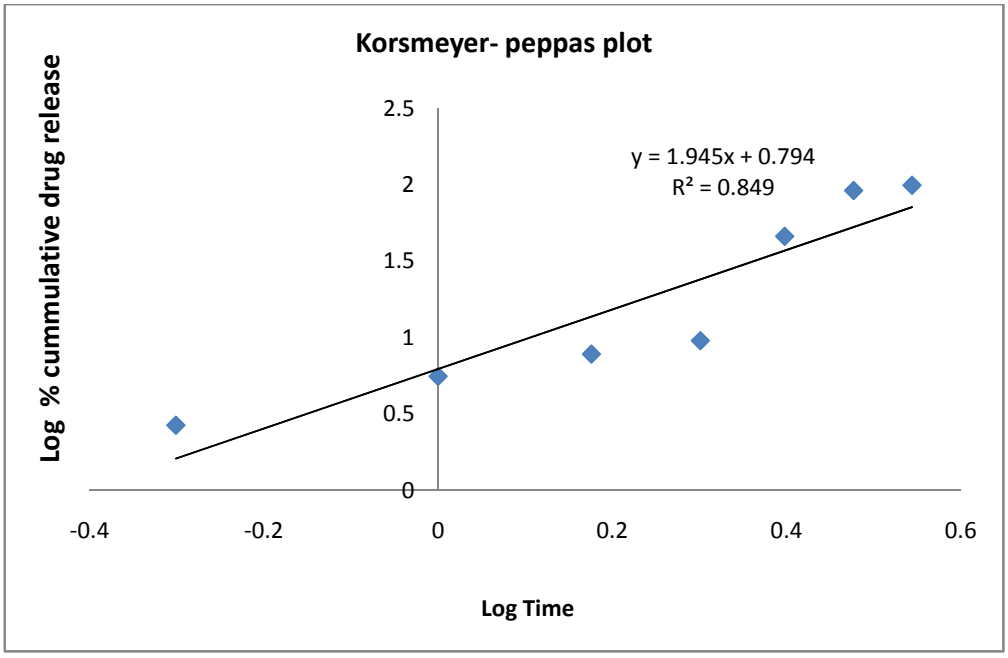


**Figure - 36**

**Evaluation Parameters of Gel**

| Formulation code | pH | Viscosity Centipoises (Cps) | Drug Content (%) | Spreadability Centimeters (Cm) | Extrudability |
|---|---|---|---|---|---|
| PTG | 6.8 | 4200 | 86 | 7.2 | E |
| MTG | 6.9 | 42000 | 72 | 7.6 | E |

**Table-41**

**Invitro Drug release profile of PTG**

| S.No | Time | Absorbance | Conc | Amt Release | %Amt Release |
|---|---|---|---|---|---|
| 1 | 15 | 0.0623 | 1.748387 | 0.699355 | 6.993548 |
| 2 | 30 | 0.0814 | 2.980645 | 1.192258 | 11.92258 |
| 3 | 45 | 0.1011 | 4.251613 | 1.700645 | 17.00645 |
| 4 | 60 | 0.1126 | 4.993548 | 1.997419 | 19.97419 |
| 5 | 90 | 0.1391 | 6.703226 | 2.68129 | 26.8129 |
| 6 | 120 | 0.1681 | 8.574194 | 3.429677 | 34.29677 |
| 7 | 150 | 0.1879 | 9.851613 | 3.940645 | 39.40645 |
| 8 | 180 | 0.2014 | 10.72258 | 4.289032 | 42.89032 |
| 9 | 240 | 0.2201 | 11.92903 | 4.771613 | 47.71613 |
| 10 | 300 | 0.2349 | 12.88387 | 5.153548 | 51.53548 |
| 11 | 360 | 0.2414 | 13.30323 | 5.32129 | 53.2129 |
| 12 | 420 | 0.2479 | 13.72258 | 5.489032 | 54.89032 |
| 13 | 480 | 0.2591 | 14.44516 | 5.778065 | 57.78065 |
| 14 | 540 | 0.2683 | 15.03871 | 6.015484 | 60.15484 |
| 15 | 600 | 0.2767 | 15.58065 | 6.232258 | 62.32258 |
| 16 | 660 | 0.2799 | 15.7871 | 6.314839 | 63.14839 |
| 17 | 720 | 0.2679 | 15.0129 | 6.005161 | 60.05161 |

**Table-42**

**Invitro Drug Release of PTG**

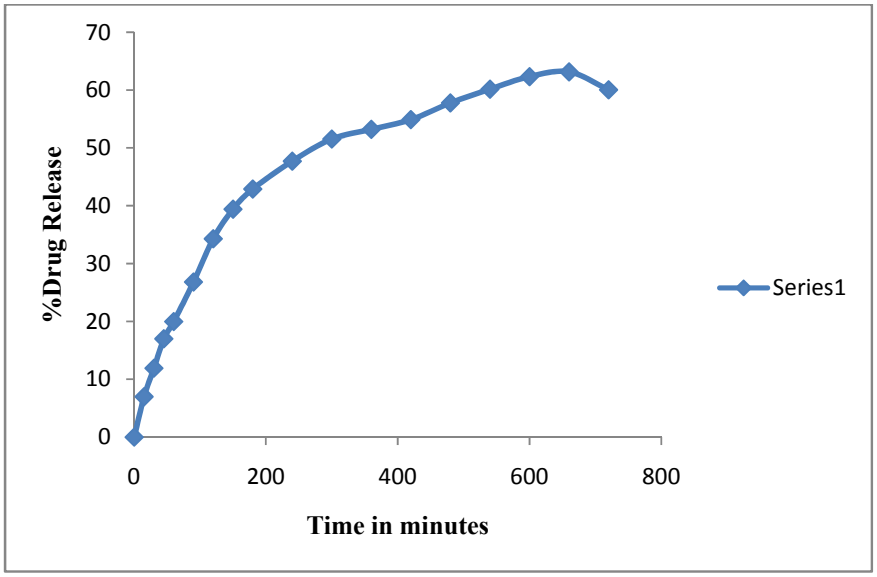


**Figure-37**

**Invitro Drug Release Profile of MTG**

| S.No | Time | Absorbance | Conc | Amt Release | %Amt Release |
|---|---|---|---|---|---|
| 1 | 15 | 0.0971 | 3.993548 | 1.597419 | 15.97419 |
| 2 | 30 | 0.1371 | 6.574194 | 2.629677 | 26.29677 |
| 3 | 45 | 0.1571 | 7.864516 | 3.145806 | 31.45806 |
| 4 | 60 | 0.1936 | 10.21935 | 4.087742 | 40.87742 |
| 5 | 90 | 0.2361 | 12.96129 | 5.184516 | 51.84516 |
| 6 | 120 | 0.2671 | 14.96129 | 5.984516 | 59.84516 |
| 7 | 150 | 0.2891 | 16.38065 | 6.552258 | 65.52258 |
| 8 | 180 | 0.2988 | 17.00645 | 6.802581 | 68.02581 |
| 9 | 240 | 0.2532 | 14.06452 | 5.625806 | 56.25806 |
| 10 | 300 | 0.2053 | 10.97419 | 4.389677 | 43.89677 |
| 11 | 360 | 0.1783 | 9.232258 | 3.692903 | 36.92903 |
| 12 | 420 | 0.0836 | 3.122581 | 1.249032 | 12.49032 |
| 13 | 480 | 0.0471 | 0.767742 | 0.307097 | 3.070968 |
| 14 | 540 | 0.0036 | -2.03871 | -0.81548 | -8.15484 |
| 15 | 600 | 0.0018 | -2.15484 | -0.86194 | -8.61935 |

**Table-43**

**Invitro Drug Release Profile of MTG**

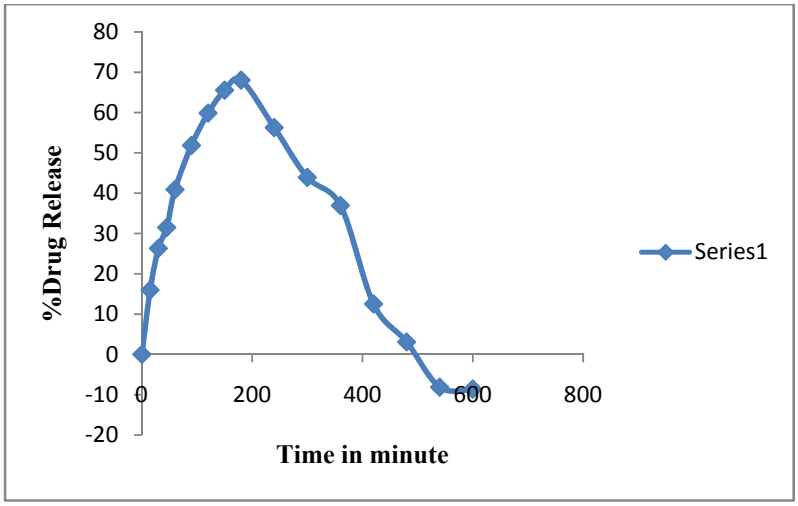


**Figure-38**

**Comparative % Drug Release Profile of PTG and MTG**

| S.No | Time | PTG | MTG |
|---|---|---|---|
| 1 | 15 | 6.993548 | 15.97419 |
| 2 | 30 | 11.92258 | 26.29677 |
| 3 | 45 | 17.00645 | 31.45806 |
| 4 | 60 | 19.97419 | 40.87742 |
| 5 | 90 | 26.8129 | 51.84516 |
| 6 | 120 | 34.29677 | 59.84516 |
| 7 | 150 | 39.40645 | 65.52258 |
| 8 | 180 | 42.89032 | 68.02581 |
| 9 | 240 | 47.71613 | 56.25806 |
| 10 | 300 | 51.53548 | 43.89677 |
| 11 | 360 | 53.2129 | 36.92903 |
| 12 | 420 | 54.89032 | 12.49032 |
| 13 | 480 | 57.78065 | 3.070968 |
| 14 | 540 | 60.15484 | -8.15484 |
| 15 | 600 | 62.32258 | -8.61935 |
| 16 | 660 | 63.14839 | |
| 17 | 720 | 60.05161 | |

**Table-44**

**Comparative Drug Release profile of PTG and MTG**

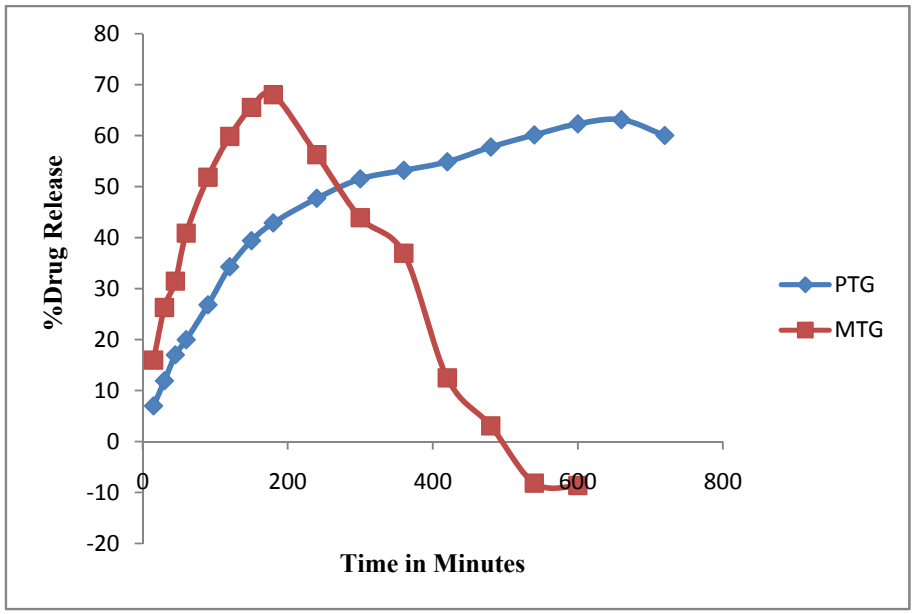


**Figure-39**

**Skin Irritation Studies**

**Skin Irritation Results**

| Formulation | Skin irritation |
|---|---|
| Placebo Gel | - |
| Phytosome Gel | - |

+ indicate edema, - indicate no edema

**Table-45**

**Preparation of Skin irritation test**

**Figure-40**

**After 24 hours study with Phytosome loaded Gel base**

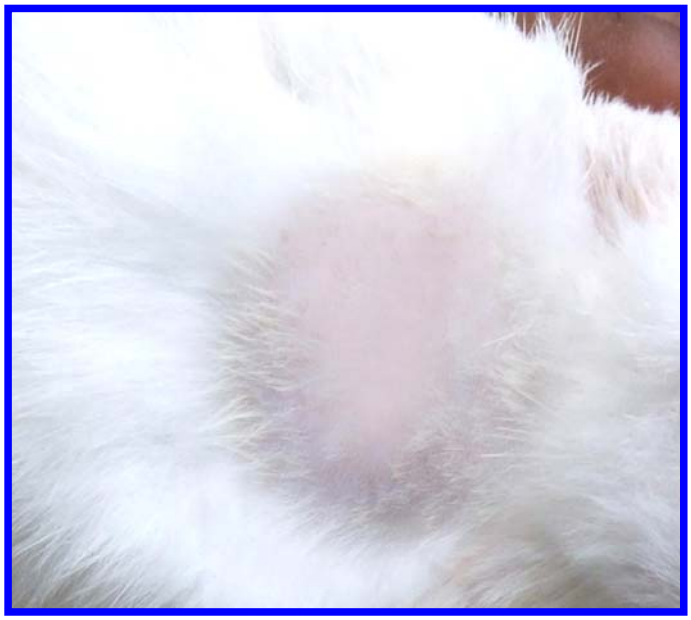

**Figure-41**

**After 24 hours study with Placebo Gel base**

**Figure- 42**

**Effect on storage on particle size**

| S.No | Time intervals | Particle Size(nm) | |
|---|---|---|---|
| | | Storage Conditions | |
| | | 40°C±2°C RH 70% | Room Temperature |
| 1 | Initial | 120 | 118 |
| 2 | After 1 month | 108 | 106 |
| 3 | After 2 month | 117 | 110 |
| 4 | After 3 month | 125 | 118 |

**Table –46**

**Effect of Storage on Percentage Drug loading**

| S.No | Time intervals | Percentage Drug Loading | |
|---|---|---|---|
| | | Storage Conditions | |
| | | 40°C±2°C RH 70% | Room Temperature |
| 1 | Initial | 64±0.5 | 62±0.5 |
| 2 | After 1 month | 58.5±0.26 | 60.5±0.36 |
| 3 | After 2 month | 54.5±1.24 | 58.5±1.04 |
| 4 | After 3 month | 52.5±1.06 | 56.5±1.01 |

**Table-47**

**Effect Storage on Percentage Drug Content**

| S.No | Time Intervals | Percentage Drug Content | |
|---|---|---|---|
| | | Storage Conditions | |
| | | 40°C±2°C RH 70% | Room Temperature |
| 1 | Initial | 78 | 78 |
| 2 | After 1 month | 73.5 | 75.6 |
| 3 | After 2 month | 63.5 | 73.5 |
| 4 | After 3 month | 58.72 | 71.62 |

**Table-48**

**Effect of Storage on Physical Stability**

| S.No | Parameters | 40°C±2°CRH 70% | Room Temperature |
|---|---|---|---|
| **1** | **Visual appearance** | | |
| | Initial | Cloudy | Cloudy |
| | 1st month | Cloudy | Cloudy |
| | 2nd month | Cloudy | Cloudy |
| | 3rd month | Cloudy | Cloudy |
| **2** | **Shape** | | |
| | Initial | Spherical | Spherical |
| | 1st month | Spherical | Spherical |
| | 2nd month | Spherical | Spherical |
| | 3rd month | Spherical | Spherical |
| **3** | **pH** | | |
| | Initial | 6.8 | 6.8 |
| | 1st month | 6.8 | 6.8 |
| | 2nd month | 6.8 | 6.8 |
| | 3rd month | 6.8 | 6.8 |
| **4** | **Phase separation** | | |
| | Initial | Not found | Not found |
| | 1st month | Not found | Not found |
| | 2nd month | Not found | Not found |
| | 3rd month | Not found | Not found |
| **5** | **Leakage** | | |
| | Initial | Not found | Not found |
| | 1st month | Not found | Not found |
| | 2nd month | Found | Not found |
| | 3rd month | Found | Not found |
| **6** | **Odor** | | |
| | Initial | Odorless | Odorless |
| | 1st month | Odorless | Odorless |
| | 2nd month | Odorless | Odorless |
| | 3rd month | Odorless | Odorless |

**Table-49**

**RESULTS AND DISCUSSION**

The Phytosomal preparation of *Thuja occidentalis* were prepared by Thin Film Hydration Technique using Rotary Flask Evaporation method.

The collected plant *Thuja occidentalis* was authenticated by Prof. P.Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Centre, Chennai. Then it was subjected to Drying and powder formation for Soxylation method using Soxhlet apparatus.

Based on the literature survey, the solvent Pet ether and Ethanol were selected for extraction.

The crude ethanolic extract of *Thuja occidentalis* was investigated for phytoconstituent identification. It confirms the presence of Corbohydrate, Alkaloid, Glycoside, Saponins, Fixed oil, Fats and Flavonoids.

Also phytochemical analysis confirms the absence of Phytosterol.

The major constituent of extract containing flavonoids was confirmed by taking absorption maxima in UV spectrometer using phosphate buffer 6.8 as solvent. It showed absorption maxima at 361nm. This was compared with reference standard of *Thuja occidentalis* constituent of flavonoid like quercetin showing the maximum absorption peak at 364 nm in the phosphate buffer 6.8 solution. This confirms the presence of Flavonoids in the crude extract.

The various formulation and process variables were optimized to get small spherical shaped vesicles with high Drug entrapment efficiency. The results are as follows:

Phosphotidyl choline and Cholesterol were used to achieve maximum drug encapsulation. The amount of phosphotidyl choline and cholesterol has an effect on the preparations of phytosome.

When the concentration of lecithin and cholesterol is too low, the DEE is also very low.

The various concentration of phosphatidyl choline were used to access the effect on the formulation characteristic from 1 to 3 ratios i.e by increasing the concentration from 40mg to 60 mg.

Increase in the concentration of phosphatidyl choline leads to more number of phytosomal dispersion, resulting in to an increased % drug entrapment.

However, Further increase in the phosphatidyl choline concentration has no proportionate increase in % drug entrapment due to large surface occupied by phophatidyl choline layer and also may be due to system saturation approaching.

When the concentration of phosphatidyl choline is too high, the DEE is very low and the release of the drug from vesicle is also affected i.e 1:1.5:6 (Drug:Cholesterol:Phosphotidyl choline).

By increasing the phospholipid to optimum concentration i.e 1:1.5:5 (Drug:Cholesterol:Phosphotidyl choline) the particle size decreases from 300 nm to 100nm and the entrapment efficiency was also increased from 64% to 82%.

Thus the optimized ratio of Cholesterol & Phosphatidyl choline to Drug was 1:1.5:5

At medium speed of (100±5rpm) The Phytosomes produced were of desired size range with maximum encapsulation efficiency. At low speed (50±5 rpm) it forms clumps and not uniform and larger size with low encapsulation efficiency. Which at high speed (above 100 rpm) the particles are irregular shaped with less encapsulation efficiency.

The temperature during phytosomal formulation was optimized. At the temperature of 60 C±2 C, spherical vesicles are formed. At high temperature above 100 C, those formed vesicles are less and irregular. At temperature below60 C, irregular shaped vesicles are formed.

Ultrasonication time was also optimized to get small spherical vesicles. At 10 minutes incomplete and large irregular shaped vesicles with high encapsulation efficiency was obtained and further increase in sonication time resulted in to reduction in % drug entrapment. The decrease in drug entrapment due to leakage of drug during sonication. Thus at 30 minutes, the vesicles are broken with leakage of drug and low encapsulation efficiency. Hence sonication time was optimized to 15 minutes and further reduction in size by increasing sonication time was not attempted.

During ultrasonication, the temperature maintenance was also optimized. At the temperature of 10 C to 20 C numerous vesicles of moderate size are formed. At low temperature 5 C to 10 C, the size of vesicle are decreases. While room temperature large sized vesicles was formed.

The effect of hydration liquid on drug entrapment efficiency was optimized. When the usage of water as a hydration liquid, the entrapment efficiency is high. While at the usage of 0.1M HCL, the DEE is very low. But DEE is achieved minimally by usage of PBS 6.8 as hydration liquid.

The morphological studies were performed by SEM, Showed uniform shape, discreate particle without aggregation and smooth in surface morphology with size range of 50nm-300nm

IR studies indicated that the extract of *Thuja occidentalis* was dispersed in molecular level. The IR spectrum of Thuja occidentalis showed sharp peaks. However the sharp peaks were disappeared in *Thuja occidentalis* loaded phytosomal formulation, it may be due to the drug can be existed as a molecular dispersion and similar functional groups and structure may broaden the peaks in phytosomal formulation.

There are no extra peaks seen other than the normal peak in the spectra of the *Thuja occidentalis* loaded Phytosomal formulation and so there is no interaction with the drug and Excipients, it confirms that they are compatible with each other.

The IR spectra of the extract and excipients combination were compared with the spectra of the crude extract and individual excipients in which no shifting of peaks was significantly found, indicating the stability of the drug during encapsulation process.

The invitro release profile of phytosomal formulation of *Thuja occidentalis* are $F_1$, $F_2$, $F_3$ were shown in Figure-24 and Table-39.

The release behavior of Drug from the Phytosomal formulation exhibited a biphasic pattern, that is characterized by faster onset of action and slower sustained release.

The Formulation $F_2$ containing the ratios of 1:1.5:5 (Drug:Cholesterol:Phosphotidyl choline) showed the drug release of 64% at 11 hours. $F_1$ showed the release of 54% at 11 hours and, $F_3$ showed 53% release at 11 hours. An initial burst drug release was observed up to 6 hours and there is a constant drug release was observed up to 12 hours.

The initial burst release may be due to the drug present in the surfacial drug on the surface of Phytosome.

The data obtained from In-vitro release profile after 12 hours was fitted with various kinetic equations to determine the mechanism of drug release and release rate as indicated by higher correlation coefficients($r^2$). The drug release from phytosomal formulation follows zero order and non-fickian diffusion.

These findings indicates that the drug release from the formulated Phytosome were diffusion controlled

To confirm the release mechanism, the data of first 60% release were applied to Korsmeyer peppas equation to find out the release exponent 'n', which indicates the mechanism of drug drug diffusion from the phytosomal formulation. The data were fitted with equation as indicated by higher correlation coefficients($r^2$) and mechanism was found to be non-fickian diffusion (anomalous transport).

Cumulative % drug release was analyzed using PRISM software. The kinetic data of F2 formulation was best fitted to Korsmeyer and Peppa's model and the value of regression co efficient, r = 0.994 and n = 0.925 which follows non-fickian, Super case-II transport.

For the best formulation F2 were subjected to skin irritation studies and stability studies at 40 C/ 75% RH up to 3 months. The potency Phytosome under accelerated stability conditions were within 90% to 110% of the label claim. Overall, results from the stability studies indicated that the beads were chemically stable for more than 3 months.

**Skin irritation studies**;

Skin irritation property of Phytosomal *Thuja occidentalis* Gel (PTG) is performed in rabbit animal model against placebo gel (PG) used as a control.

After 24 hours of application the surface of was cleaned with cotton swap and observed for allergic and hypersensitivity ( erythma and oedema)

**Stability Testing**

The selected F2 formulation is incorporated in gel base and it is evaluated for its stability by using accelerated stability chamber (40°C±2°C RH 70%) and it is compared with formulation stored in Room temperature. The results are shown in Table- 46,47,48,49.

The particle size analysis of PTG stored in AST chamber was evaluated at initial and at the end of $1^{st, 2nd}$, $3^{rd}$ month, showing 118nm, 108nm, 117nm, 125nm respectively. Also the sample stored in room temperature was evaluated the for the same period showed 100nm, 106nm, 110nm, 118nm respectively.

No much differences was observed during this period on particle size, the formulations retains with its stability.

The drug loading property is also evaluated for initial and at the end of 1st, 2nd, 3rd month of the sample stored in AST chamber showed 64%, 58.5%, 54.5%, 52.5% respectively. Also the sample stored in room temperature was evaluated the for the same period showed 62%, 60.5%, 58.5%, 56.5% respectively.

The formulation PTG on storage of AST chamber, the drug loading capacity is reduced compared with the formulation stored in room temperature. This is due to leakage of the drug from the formulation.

The drug content analysis of AST chamber stored sample evaluated for the same period showed 78%, 73.5%, 63.5%, 58.7% respectively. Also the sample stored in room temperature was evaluated the for the same period showed 78%, 75.65, 73.5%, 71.62% respectively.

On storage on AST chamber contained formulation PTG showed reduction in drug content in the formulation compared with the sample stored in room temperature.

The physical properties of sample analysed like, visual appearance, shape, pH, phase separation and odor.

No changes was observed during storage on physical properties of the AST chamber stored gel and sample stored in room temperature except the leakage of drug from the formulation occurred in the sample stored in AST chamber at the end of 2nd, 3rd months . It is stable up to the end of 1st month.

## CONCLUSION

Phytosome are one of the novel colloidal drug delivery systems that could potentially carry out multiple tasks in a predefined sequences thus holds great promise for reaching the goal of controlled as well as site specific drug release from the vesicles across the biological membrane.

The specific properties of Phytosome such as high stability, high carrier capacity, and sustained drug release up to 120 hours enable improvements of drug bioavailability and enhanced therapeutic effect, reduction of dosing frequency. And also reduced toxic side effect since the dose is reduced drastically to very lesser amount and it is delivered to target tissue.

*Thuja occidentalis* plant was collected and it is extracted and formulated to Phytosomal formulation by using cholesterol and phosphotidyl choline by thin film hydration technique with rotary flask evaporator.

Three different formulations (F1, F2, F3) were prepared and all of them subjected to various evaluations like particle size and shape, drug content, drug entrapment, invitro release studies, skin irritation studies and stability studies.

All among the formulation, F2 showed the optimum particle size range of 100nm and spherical in shape.

The drug content and drug entrapment of formulation F2 showed 78% and 82% respectively.

The invitro release profile of formulated Phytosome were evaluated and the formulation F2 proved to be of great interest since it gives a required release of 64% for an extended period of time of 12 hours.

Further, from the release kinetic study it revealed that the drug diffusion follows zero order kinetics and non-fickian diffusion controlled (Higuchi). The selected formulation has proved the purpose as designed.

The skin irritation studies of Phytosome incorporated Thuja Gel(PTG) is evaluated against Placebo Gel, and there is no irritation symptoms like erythma/oedema

The selected formulation F2 was subjected to further studies and then it is incorporated in to Phytosomal Thuja Gel (PTG) is showed more stability profile in room temperature compared to Gel stored in AST chamber.

From the results, it may conclude that the formulation F2 has achieved the objective of converting the crude extract in to a novel targeted drug delivery system as Phytosome to treat a medically challengeable disease Wart.

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